AFTERPOTENTIALS IN THE RETINA OF *DROSOPHILA*

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This thesis was composed by myself. The experimental work in chapter 2 was done in collaboration with Derek Cosens, and part of chapter 2 has been published as a paper (Wright and Cosens, 1977). This paper is bound at the end of the thesis. The remainder of the thesis is my own work.

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ABSTRACT

This thesis examines extracellular afterpotentials in the retina of Drosophila melanogaster. The results presented, and comparison of these with the available intracellular recordings, indicate that the ERG of Drosophila is a scaled down version of the transmembrane potentials of classes of retinula cells. The afterpotentials investigated are due to retinula cells R1-R6.

Afterpotentials in photoreceptors are known to be associated with bi-stable visual pigments. Evidence is presented which suggests that the prolonged depolarising afterpotential (PDA) of R1-R6 in Drosophila results from a component of the membrane conductance of these cells which is correlated with the quantity of metarhodopsin (M580) generated by a stimulus.

A simulation, using a membrane model and assuming the M580-conductance, predicts a number of the experimental findings: for example, a high negative correlation between the ERG d.c. potential and the size of the ERG response to a testflash, and a sigmoidal decline of the d.c. potential when M580 is eliminated by long-wavelength light. The simulation also predicts effects upon the classic V-log I curve which are detected in the Calliphora data of Razmjoo and Hamdorf (1976).

Some of the phenomena of the PDA reported in other species (Limulus and Balanus) can only be replicated in Drosophila after small doses of blue light which induce "brief afterpotentials". These phenomena (decay of the afterpotential independently of the decay of metarhodopsin and suppression of the afterpotential by previous metarhodopsin to rhodopsin conversion) have been taken by others as evidence for an "excitor-inhibitor" model of phototransduction. The

value of this model is questioned and a biophysical model of the events in the Drosophila photoreceptors is presented.

The afterpotential is shown to occur in wildtype (red eyed) Drosophila, and to have behavioural consequences.

A model of extracellular potentials based on capacitance is advanced. This may shed light on electrical potentials in extracellular compartments such as the retina of insects.

CONTENTS

CHAPTER 1

INTRACELLULAR AND EXTRACELLULAR AFTERPOTENTIALS IN THE RETINA OF *DROSOPHILA*

1: Introduction

Vision is useful to animals in many ways, for instance in the selection of habitats, the location of mates, the detection of predators and orientation in space (Brown, 1975). One of the characteristics of sensory systems in general is their ability to obtain useful information about the environment over a wide range of stimulus intensities. This is a difficult task for a linear system. Animals have solved this problem in two ways, (1) the basic response of sensory receptors is non-linear and (2) the range of operation of the receptor alters to suit the input, a process called adaptation. The mechanisms involved in both of these processes are poorly understood. This thesis is about afterpotentials in the retina of *Drosophila* which prove to reflect alterations in the performance of classes of photoreceptors. Whether this change in performance, or adaptation, is "adaptive" in an evolutionary sense is not clear, but an understanding of its mechanism may offer an insight into the general problems of the response and the plasticity of sensory cells.

The visual systems of invertebrates, particularly of insects, and among the insects particularly those of flies, have been subject to investigation for many years. A number of recent symposium

volumes indicate current interest in the field (Langer, 1972; Wehner, 1972; Horridge, 1974; Snyder and Menzel, 1975). The field can be neatly divided into anatomical, physiological and behavioural subdisciplines. Goldsmith (1973) is an excellent general review; Goldsmith (1964) and Goldsmith and Bernard (1974) are reviews devoted to the insect visual system and Pak (1975) and Pak and Pinto (1976) are reviews which contain large sections specifically concerned with work relating to the fruitfly Drosophila melanogaster which is the subject of this thesis.

At its periphery the visual system of dipteran flies comprises a shallow layer of photoreceptors lying proximal to the corneal surface of the compound eye: this layer is called the peripheral retina. The photoreceptor cells lying in the retina consist of large cell bodies, and small rod-like structures which are perpendicular to the corneal surface and are called rhabdomeres. The photoreceptor cells are primary sensory neurons and their axons pass proximally to higher order ganglia: the lamina ganglionaris and the medulla (Trujillo-Cenoz, 1965; Braitenberg, 1967). Three dorsal ocelli complete what is known of the input to the visual system of dipteran flies (reviews in Goldsmith and Bernard, 1974, Pak, 1975, Pak and Pinto, 1976) Microstructural details are described below and illustrated in Fig. 1.

Studies of the visual behaviour of Drosophila have covered phototaxis (e.g. Harris et al., 1976; Jacob et al., 1977), colour vision (e.g. Schuemperli, 1973; Menne and Spatz, 1977) and analyses of visual acuity and the performance of movement detection tasks (for a comprehensive review and revision of previous interpretations see Heisenberg and Buchner, 1977). The behavioural work will be described more fully at the end of this chapter and in chapter 5.

Figure 1: A schematic diagram illustrating the gross anatomy of the visual system of Drosophila and the numbering system for the retinula cells (from Heisenberg and Buchner, 1977). In the recording method used in this thesis an electrode was placed in the retinula cell (Rc) region of the eye (the peripheral retina). The electrical potential between such an electrode and an electrode in the haemolymph of the fly is called the ERG. Despite the location of the electrodes, the ERG includes electrical responses which originate in the lamina (La). Figure 2 shows the type of record that can be obtained using the ERG recording technique.

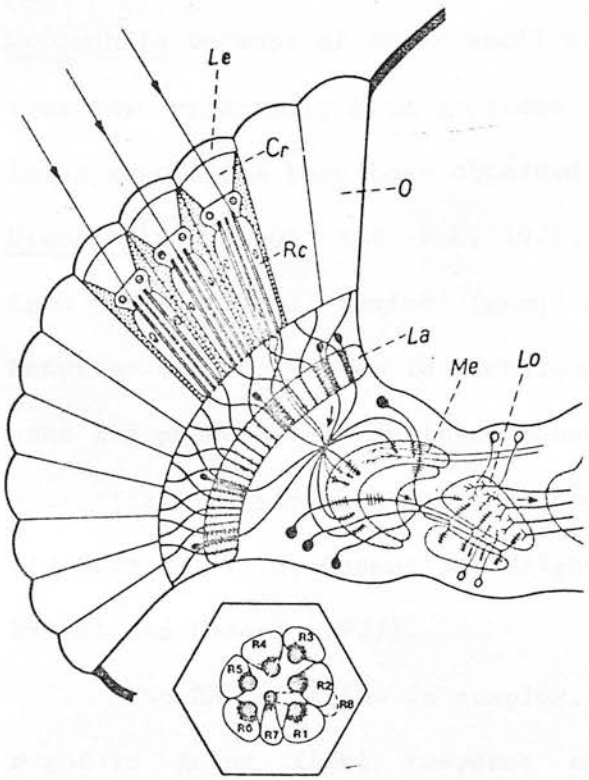
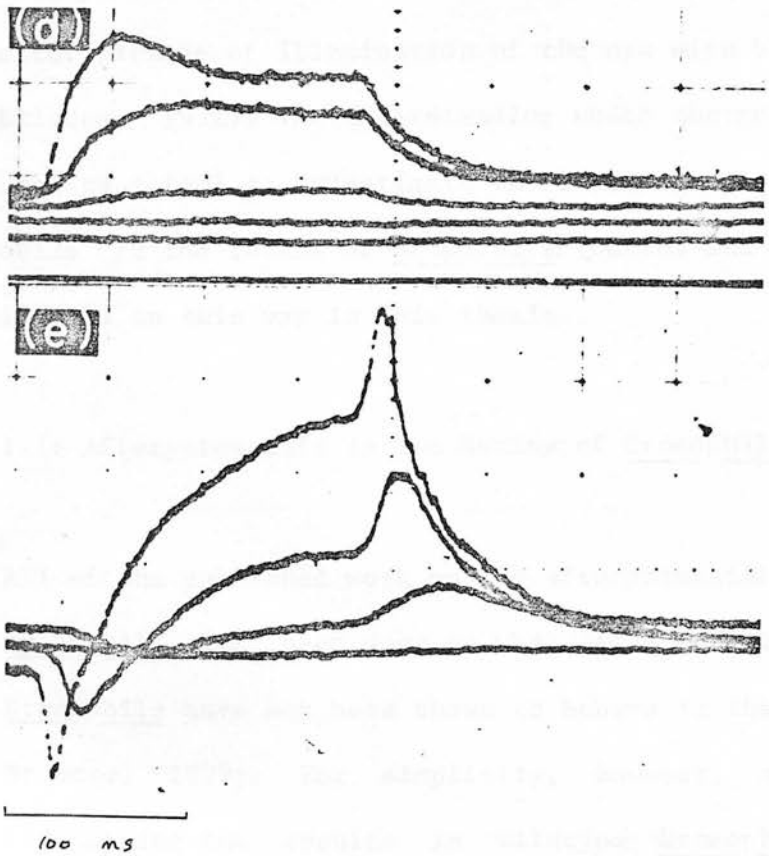


Fig. 1. Schematic drawing of the dipteran eye and optic ganglia; *Cr*, Crystalline cone; *La*, Lamina; *Le*, Lenslet; *Lo*, Lobula; *Me*, Medulla; *O*, Ommatidium; *Rc*, Receptor cell (after Kirschfeld, 1971). Inset figure: crossection of retinula cells in one ommatidium

Physiological workers have concentrated on investigation of the electrical events occurring - largely - in the peripheral retina, or photoreceptor layer of the eye, and the biochemical basis of these events. Most of the electrical studies have made use of a gross extracellular electrical potential which can be measured between the peripheral retina and the general haemolymph of the fly, and which alters according to the illumination applied to the eye. This potential is called the electroretinogram and is abbreviated to ERG. The ERG is easily measured and has been used extensively in attempts to characterise to performance of the visual system or in screening techniques to isolate visually defective mutants (see Heisenberg and Goetz, 1975; Pak, 1975). Larger diptera have also been examined by measuring the electrical potential inside individual photoreceptor cells (e.g. Calliphora: Washizu, 1964). Similar intracellular recordings are reportedly difficult to obtain from the photoreceptors of Drosophila because of their small size. But recently these difficulties have apparently been overcome, and at least occasional intracellular recordings have been obtained from cells in the retina of Drosophila (Alawi and Pak, 1971; see Pak and Pinto, 1976) and even from cells in the lamina (ganglionaris) (Alawi and Pak, 1971). Despite these advances in technique, the ERG remains the most widely used and productively employed measure in the investigation of the electrical performance of the Drosophila visual system (e.g. Cosens and Briscoe, 1972; Cosens and Wright, 1975; Harris et al., 1976; Wright and Cosens, 1977).

The ERG of flies is complex, being composed of a sustained negative going light response upon which are superimposed rapid transient potentials at the initiation and the termination of a stimulus (see Fig. 2; Cosens and Wright, 1975). In the attempt to

Figure 2: These records illustrate the complex ERG response of Drosophila which includes fast lamina transients when the stimulus goes on and when it goes off (e). After blue-adaptation the response is monophasic, that is the lamina trasients are absent (d). (From Cosens and Wright, 1975). Each picture shows responses to stimuli which varied in intensity over a range of 5 log units.



determine the cellular origin of the ERG a number of techniques have been used: these are reviewed in Goldsmith (1964) and Goldsmith and Bernard (1974). The slow sustained negative going response is believed to arise due to the activity of the photoreceptor cells in the retina; the rapid transients have been shown to arise due to the activity of cells in the first optic ganglion, the lamina (extracellular evidence: Heisenberg, 1971; intracellular recordings: Alawi and Pak, 1971).

A much firmer and fuller understanding of the origin of the ERG has arisen from the interpretation of prolonged depolarising afterpotentials which arise in the retina of white-eyed Drosophila after periods of illumination of the eye with blue light (Cosens and Briscoe, 1972). The understanding which emerges allows the ERG to be used as a tool to investigate the behaviour of individual classes of cells in the retina of Drosophila (Cosens and Wright, 1975). The ERG is used in this way in this thesis.

1.1: Afterpotentials in the Retina of Drosophila

All of the published work on the afterpotentials in the retina of Drosophila has been done on white-eyed mutants; wildtype (red-eyed) Drosophila have not been shown to behave in the same way (Cosens and Briscoe, 1972). For simplicity, however, and because a method of replicating the results in wildtype Drosophila is described in chapter 5, the designation "white-eyed" is generally omitted below.

Long-term changes in the resting potential across the membranes of classes of cells in the retina of Drosophila melanogaster can be inferred from long-term changes in extracellular and intracel-

lular potentials, recorded relative to a remote electrode, say, in the thorax of the fly (ERG: Cosens and Briscoe, 1972; intracellular: Minke et al., 1975).

Blue light induces prolonged potential changes corresponding to the depolarisation of the transmembrane potential of a class of cells in the eye. This depolarising change in resting potential, or depolarising afterpotential, may last for several hours after the termination of the blue stimulus, but the resting potential is restored to its initial value by orange light (Cosens and Briscoe, 1972). This "blue/orange" effect has been attributed to changes in the resting potential across the membranes of retinula cells R1-R6 in the retina (see Fig. 1 for the cell nomenclature; Cosens and Wright, 1975; Minke et al., 1975; Harris et al., 1976).

1.2: Evidence for the Neural Origin of the Blue Light Induced Afterpotential

During the prolonged afterpotential in the ERG of Drosophila which follows illumination with blue light a residual monophasic response to light remains (Cosens and Briscoe, 1972 see Fig. 2). This response lacks the fast on and off transients of the typical ERG and which originate in the lamina (Heisenberg, 1971; Alawi and Pak, 1971). This monophasic response resembles the intracellular receptor potential of R1-R6 recorded by Alawi and Pak (1971) and classic intracellular recordings from dipteran photoreceptors (Calliphora: Washizu, 1964). It is likely, therefore, to be a receptor potential, but it is not likely to be that of cells R1-R6 because these cells have axons which synapse in the lamina while those of the central cells R7 and R8 pass through the lamina without synapsing (Power, 1943; Trujillo-Cenoz,

1965; Braitenberg, 1967). On this evidence Cosens and Wright (1975) concluded that the monophasic response is the receptor potential of the central cells R7 and R8. They tested this idea by measuring the spectral sensitivity of the monophasic response.

Dipteran retinula cells R1-R6 respond maximally around 480 to 510 nm; the central cells R7 together with R8 respond maximally around 470 nm (reviewed in Snyder and Pask, 1973; R7 is now known to be an ultraviolet receptor: Harris et al., 1976, see below). Cosens and Wright (1975) found that the dark-adapted ERG of Drosophila responds maximally around 490 to 500 nm while the monophasic response responds maximally around 460 nm: a result consistent with the idea that the dark-adapted ERG is dominated by the response of cells R1-R6 while the monophasic response is dominated by cells R7 and R8, and that the activity of R1-R6 is suspended during the blue light induced afterpotential. They also found that the sensitivity of the monophasic response was two logarithmic units lower than that of the dark-adapted ERG: a result which is consistent with high and low sensitivity measurements of the spectral sensitivity of Drosophila obtained in behavioural experiments by Schuemperli (1973).

These data indicate that R1-R6, which have high absolute sensitivity, may be rendered depolarised and unresponsive by an afterpotential induced by blue light, while the central cells R7 and R8, which have low absolute sensitivity, are probably not subject to a blue light induced afterpotential and remain responsive.

Minke et al. (1975) independently obtained similar data and came to the same conclusions as Cosens and Wright (1975). In addition they were successful in recording a blue light induced afterpotential using intracellular recording techniques in 29 out of 30 penetrated cells in the retina. While this high ratio of successful recordings

of the afterpotential seems to imply that all of the photoreceptors in the retina respond to blue light with an afterpotential, Minke et al. regard it as most likely that the 29 successful recordings were from the R1-R6 cell class. Unfortunately the penetrated cells were not identified histologically by dye injection.

Further evidence that the prolonged effects of blue light upon the photoreceptors of the Drosophila compound eye are confined to R1-R6 was obtained by Cosens (1976). He found that prolonged exposure of white-eyed Drosophila to blue light results in degeneration of the structure of the rhabdomeres of cells R1-R6 but not of those of R7 or R8. Yellow light did not induce degeneration in any of the retinula cells (Cosens, 1976). Together with the previously described evidence, this result suggests that the visual pigment of R1-R6 differs from that of the central cells R7 and R8. Cosens' data indicate that the extended presence of a photoproduct of blue light in R1-R6 of Drosophila directly induces membrane instability which leads to degeneration.

The results obtained by Cosens and Wright (1975) and Minke et al. (1975) have been thoroughly corroborated by Harris et al. (1976) using morphological mutants in which R1-R6 are absent (reviewed in Pak and Pinto, 1976; see below).

An additional set of events, essentially similar to the blue/orange effects described above, has been described by Minke et al. (1975) and Harris et al. (1976). In this case the afterpotental is induced by ultraviolet (UV) light and can be eliminated by blue light. This "UV/blue" effect can be attributed to changes in the resting potential across the membranes of one of the central cells: R7 (Harris et al., 1976).

Both the blue/orange and the UV/blue effects in the Drosophila

retina are characterised by prolonged changes in the resting potential of sensory neurons. And in both cases these electrical events are associated with photoconversions occurring within a bi-stable visual pigment system.

1.3: Bi-Stable Visual Pigment Systems in Drosophila

Two classes of retinula cells in the compound eye of Drosophila contain a visual pigment which may exist in either of two stable but photoconvertible states (see below for a comparison of bi-stable visual pigment systems with the vertebrate visual pigment system). These two classes of cells are the six peripheral retinula cells of each ommatidium: R1-R6, and one of the central retinula cells: R7, in which prolonged depolarising afterpotentials occur. Together these two classes of cells account for seven eighths of the sensory cells in the retina of the fly.

The two states of the visual pigment molecule within both of these classes of retinula cells absorb light maximally at different wavelengths. For instance in R1-R6 the state of the visual pigment molecule found in a completely dark-adapted retina absorbs light maximally at 480 nm and is called rhodopsin R480. Irradiation of this visual pigment with blue light (approximately 480 nm) yields a molecule which absorbs maximally at 580 nm and is called metarhodopsin M580. Irradiation of M580 with orange light (approximately 580 nm) yields the rhodopsin R480 again (Pak and Lidington, 1974; Ostroy et al., 1974; Harris et al., 1976).

The positions of the absorbance maxima of the bi-stable visual pigment of R1-R6, the stability of the metarhodopsin, and the way in which the two states of the visual pigment may be interconverted by

blue and orange light strongly implicate interconversions of the rhodopsin R480 and its metarhodopsin M580 in the causation of the blue/orange effect electrical events described above (Pak and Lidington, 1974; Cosens and Wright, 1975; Stark, 1975; Minke et al., 1975; Harris et al., 1976). The action spectra for the blue/orange effect are consistent with the absorption spectra of this visual pigment pair (Stark, 1975) and the action spectra of the UV/blue effect are consistent with the absorption spectra of the bi-stable visual pigment in R7 (Harris et al., 1976).

Harris et al. (1976) characterised a series of morphological mutants of Drosophila in which whole classes of retinula cells are absent. Their physiological studies with these mutants (a) confirm that the blue/orange effect is due to R1-R6, (b) localise the UV/blue effect to R7 and (c) allow identification of the visual pigments of R1-R6 and R7. Physiological studies with mutant animals always raise problems because of the possibility of pleiotropic effects caused by the mutant gene. In this case, however, the close correspondence of results obtained by the use of mutants with the purely physiological results described above eliminates this as a major problem.

The compound eye of Drosophila comprises some 800 ommatidia - structural units of the eye, each lying beneath a single corneal lens - each of which contains, in addition to accessory structures and cells, eight photoreceptors with individual rod-like rhabdomeres. The rhabdomere is formed from microvillar projections of the cell membrane, and contains the visual pigment (Langer and Thorell, 1966). The photoreceptor, or retinula, cells are arranged in an asymmetric fashion and the rhabdomere of each retinula cell is separate from those of the others so that they can be resolved microscopically in the intact fly (Franceschini, 1975). The organisa-

tion of the retinula cells and the system used for identifying them are indicated in Fig. 1. The position of the rhabdomeres, not the cell bodies, governs the naming of the cells. Retinula cells R1-R6 have rhabdomeres which lie in a trapezoid array around the centrally located rod consisting of the abutted rhabdomeres of R7 and R8. The rhabdomere of R7 lies distal to that of R8 (Dietrich, 1909) which receives light guided to it by the light guide formed by the rhabdomere of cell R7 (Goldsmith and Bernard, 1974).

Harris et al. (1976) used mutants in which the rhabdomeres and/or cell bodies of R1-R6 and/or R7 are absent. Their findings are that flies in which cells R1-R6 have degenerated following exposure to light (rdgB) or in which the rhabdomeres (and hence the visual pigment) of R1-R6 are absent (ora), (a) show no blue/orange effect (b) show the UV/blue effect (c) lack the R480/M580 visual pigment dominant in flies with the full complement of photoreceptors (Ostroy et al., 1974), but (d) have an R370/M470 visual pigment. Flies in which R7 is also absent (sev rdgB or sev;ora) (a) show neither blue/orange nor UV/ blue effects and (b) show no detectable visual pigment transition (by the measurement of difference spectra). Flies lacking only R7 (sev) (a) show a blue/orange effect but (b) show no UV/blue effect and (c) contain an R480/M580 visual pigment (the values of Ostroy et al., 1974 are used here, those given by Harris et al., 1976 are marginally different). These data are consistent with the following interpretation of events which complements and extends that arising from the purely physiological studies:

1. R1-R6 contain the visual pigment identified by Ostroy et al. (1974) as an R480 with a metarhodopsin M580; they are the site of origin of the blue/orange electrical events (Cosens and Briscoe,

1972); and the blue/orange effect is in some unknown way associated with the visual pigment transitions.

2. R7 contains a newly identified visual pigment comprising an R370 and a metarhodopsin M470; it is the site of origin of the UV/blue effect (Minke et al., 1975; Stark, 1975).

3. R8 has no known afterpotential and contains an unidentified visual pigment which probably does not have two stable states.

Since R7 is a UV-receptor the blue peak in the spectral sensitivity of the central cells must represent the activity of R8.

Despite the now clear association between the bi-stable visual pigments and the depolarising afterpotentials in the Drosophila retina - and the implied similarity to the depolarising afterpotentials known in other species (see below) - it remains unclear how the visual pigment transitions are linked causally to the genesis of the afterpotential (Pak and Pinto, 1976; see chapter 2). However, because visual pigment transitions and depolarisation of retinula cells are involved in both the afterpotential mechanism and the normal light response of these photoreceptors, it is to be expected that any further understanding of the afterpotential mechanism will shed light on a central problem in sensory physiology: the mechanism of phototransduction. This thesis provides a more comprehensive description of the blue/orange afterpotential in the Drosophila retina than is currently available elsewhere. Hopefully what is described will have relevance to the more general questions of phototransduction (see chapters 2 and 4).

Pak and co-workers have combined studies of phototransduction mutants with an investigation of the afterpotential mechanism in

R1-R6 in the hope of furthering our understanding of phototransduction (Pak and Pinto, 1976).

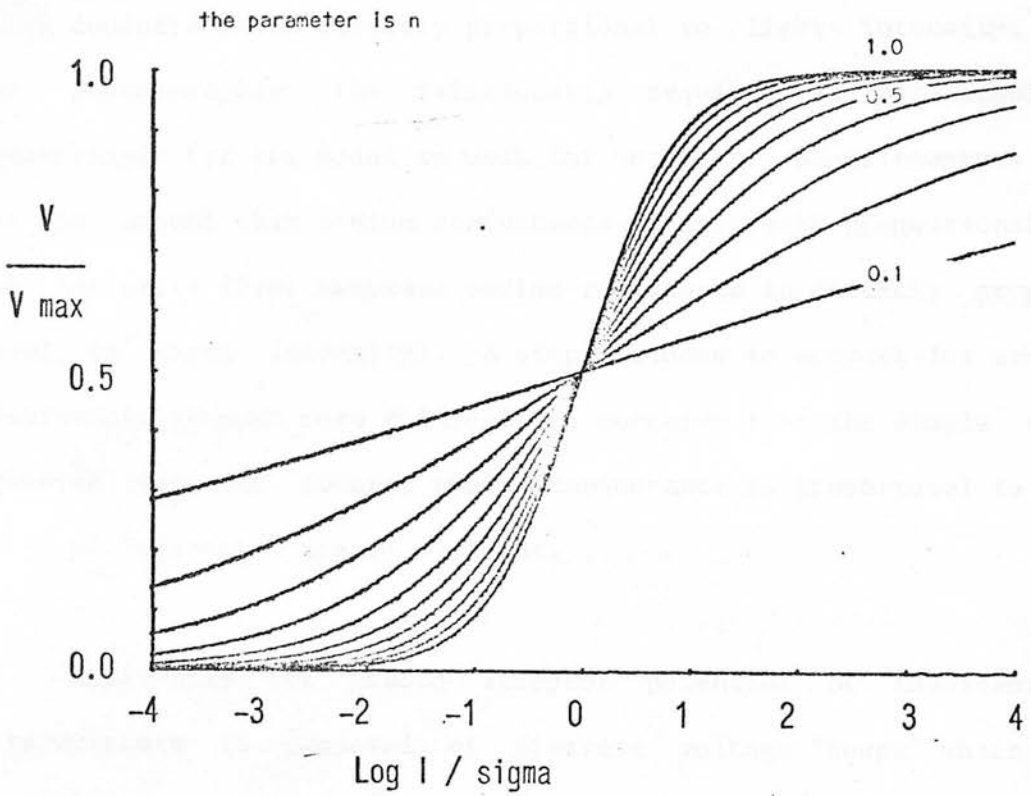
2: Phototransduction

2.1: The Electrogenic Mechanism

Photoreceptors are primary sensory neurons specialised to detect the presence of light and which generate an electrical signal: the currency of the nervous system. The electrical response which is most readily measured is the receptor potential - a change in transmembrane potential, or its products the intracellular and extracellular (ERG) potentials - which is graded according to the intensity of the light stimulus. Lipetz (1969, 1971) has summarised the evidence which shows that the output of most sensory receptors (including photoreceptors) obeys a tanh log relationship to the stimulus intensity. That is, the voltage response of a photoreceptor is sigmoidally related to the logarithm of light intensiy (Fig. 3 is a plot of the theoretical curve). The tanh log law can be derived, making certain assumptions, from the "self-shunting" membrane model of Naka and Rushton, first used to describe the vertebrate S-potential (Lipetz, 1969, 1971; Naka and Rushton, 1966).

Naka and Rushton's model closely resembles the parallel conductance model of the squid axon (see chapter 2). The model was originally intended by its authors as a simple description of their data but it is now generally accepted that photoreceptors behave like non-spiking neurons, and their model has been adopted as a biophysical model for the interpretation of the behaviour of photoreceptors as for other sensory receptors (Shaw, 1968; Sillman, 1969; Goldsmith,

Figure 3: The figure shows calculated solutions to equations similar to those of Lipetz (1969, 1971) for the relationship between the response of a sensory receptor and the intensity of the stimulus applied to it. V/Vmax is the response as a fraction of its maximum value; log I is the logarithm of the stimulus intensity. The equation reproduces the sigmoid V-log I curve which is so often reported experimentally. The parameter n must be adjusted for each receptor. It simply has the effect of stretching or compressing the basic sigmoid curve. The n-value varies between species. In Drosophila and Calliphora R1-R6 it is approximately 0.5 (Minke et al., 1975; chapter 3).



1973; Laughlin, 1975)

The membranes of photoreceptors separate ion species and the receptor potential is largely due to light initiated modulation of membrane sodium conductance. In vertebrate photoreceptors light decreases membrane sodium conductance causing a hyperpolarising receptor potential (conductance decrease: Toyoda et al., 1969; Baylor and Fourtes, 1970; involvement of sodium: Fourtes, 1959; Millechia and Mauro, 1969; Brown et al., 1970). In most invetebrate photoreceptors the movement of sodium is reversed: light increases membrane sodium conductance causing a depolarising receptor potential (Fulpius and Baumann, 1969; see Goldsmith, 1973 for comparison of the position in vertebrate and invertebrate photoreceptors, Rodiek, 1973 for a review of the vertebrate data). According to the model of Shaw (1968), advanced to account for results obtained from the locust retina, sodium conductance is directly proportional to light intensity. In other photoreceptors the relationship required is more complex. Interestingly for the model to work for vertebrate photoreceptors it must be argued that sodium conductance is inversely proportional to light intensity (i.e. membrane sodium resistance is directly proportional to light intensity). A simple scheme to account for such a relationship is much more difficult to conceive than the simple case suggested for the locust where conductance is proportonal to the number of "channels" opened by light.

Apparently the graded receptor potential of invertebrate photoreceptors is composed of discrete voltage "bumps" which are currently interpreted as responses to individual photons (Scholes, 1965; Fourtes and Yeadle, 1964; Dodge et al., 1968; Wu

and Pak, 1975 describe voltage bumps from Drosophila photoreceptors). Presumably the voltage bump results from a discrete unit of membrane conductance being opened, perhaps the opening of a single conductance channel.

2.2: The Action of Light on the Visual Pigment

Photoreceptors capture photons by absorption in the visual pigment rhodopsin. An absorbed photon causes a cis to trans isomerisation at position 11 of the chromophore retinal (Hubbard and Kropf, 1958). In vertebrate photoreceptors the visual pigment passes through a series of transient intermediate states and finally hydrolyses to a large protein molecule, opsin, and all-trans retinal (Wald, 1968). Resynthesis of rhodopsin is enzymic (comprehensive review in Brindley, 1970).

The visual pigment cycle in invertebrates is different as intimated above. All-trans retinal remains bound to the opsin in a state corresponding to metarhodopsin I in the vertebrate cycle (Goldsmith, 1973). This stable metarhodopsin is photoconvertible with rhodopsin (e.g. squid: Hubbard and St. George, 1958; Calliphora: Hamdorf and Rosner, 1973; Stavenga et al., 1973; Drosophila: Ostroy et al., 1974; Pak and Lidington, 1974; Harris et al., 1976). There appears to be no evidence for the hydrolysis of these invertebrate metarhodopsins to retinal plus opsin, but Cosens' (1975) observation of membrane instability after prolonged exposure of R1-R6 of Drosophila to blue light might be related to hydrolysis of the visual pigment molecule.

2.3: The Initiation of a Membrane Conductance Change - Are Internal

Transmitters Involved?

In vertebrate rods the visual pigment lies in the membranes of sac-like disks which are isolated from the boundary membrane of the cell across which the electrical events are recorded (Brindley, 1970). Yoshikami and Hagins (1973) have suggested that in order to carry information from the visual pigment to the cell membrane calcium ions are released from rod disks upon isomerisation of the visual pigment, and act as an internal transmitter which modulates the sodium conductance of the cell membrane. A requirement for an internal transmitter in the rhabdomeric photoreceptors of invertebrates where the visual pigment molecule lies in the membrane of microvillar extensions of the cell membrane (Langer and Thorell, 1966) is less obvious. However, Cone (1973) has advanced theoretical arguments for such an internal transmitter in rhabdomeric photoreceptors, despite evidence that the light induced conductance increase occurs at the rhabdomeres (leech photoreceptor: Lasansky and Fourtes, 1969).

Hard evidence for transmitters in invertebrate photoreceptors is not available but models of the afterpotential mechanism have been advanced which elaborate considerably upon the basic idea. Indeed the behaviour of afterpotentials has been interpreted as evidence for internal transmitters (Hochstein et al., 1973; Minke et al., 1973b; Minke et al., 1975; Muijser et al., 1975; Stark and Zitzmann, 1976; Hillman et al., 1976). Harris and Stark (1976) report that they have isolated Drosophila mutants which are defective in two antagonistic types of internal transmitter.

3.1: Afterpotentials in Photoreceptors: Phenomena and Models

The long-term resting potential changes associated with bi-stable visual pigments have been given the name "prolonged depolarising afterpotentials" (Nolte and Brown, 1972) which in the literature is commonly abbreviated to PDA. By analogy with this term used as a description of intracellular data, the term "prolonged corneal negative afterpotential" (PCNA) has been applied to extracellular records of the same phenomenon in Drosophila (Wright and Cosens, 1977). Models of the mechanism of the PDA/PCNA are summarised below.

3.2: The Nolte and Brown (1972) model of the PDA in Limulus Ultraviolet Receptors

Nolte et al. (1968) discovered that an intense ultraviolet stimulus applied to the UV receptors of the median eye of Limulus was followed by a depolarising afterpotential lasting several minutes. The receptors were repolarised rapidly by visible light to which the photoreceptors were otherwise insensitive. The spectral sensitivity of the repolarisation mechanism peaked around 480 nm (Nolte and Brown, 1972). Nolte and Brown inferred the presence of a stable metarhodopsin M480 similar to the stable invertebrate metarhodopsins with absorbance maxima at 490 to 500 nm reported to be photoconvertible with rhodopsin (octopus and cuttlefish: Brown and Brown, 1958;spider crab: Hays and Goldsmith, 1969; squid: Hubbard and St. George, 1958; lobster: Wald and Hubbard, 1957). They proposed the following model to explain their results: (a) The R360 of the ultraviolet receptor is photoconverted to a hypothetical M480 by UV light. (b) M480 is rapidly converted in darkness to R360, but the conversion system has a limited capacity. (c) R360 can be rapidly regenerated by exposure

of M480 to visible light and (d) the presence of M480 implies a patch of "light-activated" membrane.

The term "light-activated" patch of membrane, we may now interpret as a component of membrane sodium conductance. Nolte and Brown's posited visual pigment events were confirmed by measurements of the early receptor potential (ERP) by Minke et al. (1973b). Nolte and Brown, however, implied that the decay of the afterpotential occurs concurrently with the decay of M480. As they point out their data provides no evidence that this is the case. Minke et al. (1973b) report that the afterpotential in fact declines more rapidly than the decay of M480. Nolte and Brown were aware of this possibility and to encompass it suggested as an alternative to their model that the "light activated" patch of membrane is present during the transition of M480 through a conversion system (Nolte and Brown, 1972). Minke et al. (1973b), however, found that the PDA in Limulus UV receptors has a number of similarities to the PDA in Balanus photoreceptors which are not predicted by either of Nolte and Brown's models. They proposed that the PDA in Limulus UV receptors was due to an "excitor-inhibitor" interaction according to the model of Hochstein et al. (1973) which will now be described.

3.3: The "Excitor-Inhibitor" Model of the PDA in the Balanus Photoreceptor (Hochstein et al., 1973)

Minke et al. (1973a) and Hochstein et al. (1973), respectively, examined the early and late receptor potentials from the barnacle photoreceptor. Using the early receptor potential they identified a visual pigment with two thermally stable states: an R532 and an M495. The late receptor potential revealed a PDA after M to R conversion.

The PDA lasted up to 30 minutes but decayed independently of the decay of the metarhodopsin M495 which was "stable" for over 3 hours.

In addition, and decisive in the subsequent erection of their model, they found two situations in which a PDA could not be induced. (1) After the decay of a previous PDA if M495 had not had time to decay back to R532. (2) For a brief period immediately after photoregeneration of R532 (called the "anti-tail" phenomenon). Hochstein et al. (1973) erected the following ad hoc model as an explanation of their results:

1. An "excitor" molecule is responsible for the initiation of the membrane conductance change leading to depolarisation.

2. Each rhodopsin to metarhodopsin transition releases an unspecified number of "excitors".

3. An "inhibitor" molecule acts to neutralise one "excitor" molecule thus bringing about repolarisation. The "inhibitor" has no direct effect upon the cell membrane.

4. Each metarhodopsin to rhodopsin transition releases an unspecified number of "inhibitors".

5. "Excitor" and "inhibitor" molecules have long but finite lifetimes, "excitor" and "inhibitor" interact to produce both stimulus-coincident depolarisation and the PDA. A PDA marks an excess of "excitors". An "anti-tail" marks an excess of "inhibitors".

This model certainly has its attractions, the evidence for this is how widely it has been accepted and (uncritically) applied to other species. There is no doubt that with suitable mental manipulation of the parameters of this model the phenomena of the PDA can be "replicated", but this should not be surprising: it is an ad hoc model constructed to do just that. Unfortunately no independent evidence for the existence of the variables in the model has been

forthcoming. It is also regretable that its authors have chosen not to quantify the model in any way. The model thus contains a large number of, not only ad hoc, but also completely unspecified variables: nature of the molecules; lifetime of the molecules; diffusion rates, mode of action at the membrane, relative timing and quantitative relationship of the molecules and the mode of the interaction. A model with so many variables is not only likely to be able to "explain" any experimental result, but is extremely difficult to refute. This last leads to questions regarding the scientific nature of a model or hypothesis (Popper, 1972). The authors of the model have offered no experimental test which could refute the model. Despite these criticisms, the model does adequately summarise an otherwise confusing series of phenomena.

The "excitor-inhibitor" model has been widely adopted and even elaborated (e.g. Stark and Zitzmann, 1976), on the assumption that it explains the PDA phenomena in the photoreceptors of various species. In some cases, at least, it is clear that the phenomenology in the species in question is similar to that of Balanus in that two states exist from which a PDA cannot be created (e.g. Limulus: Minke et al., 1973b). In others this is not clear from the literature.

The model has been assumed in the interpretation of data from Balanus photoreceptors by Hillman et al. (1976), adopted to explain the PDA in Limulus photoreceptors by Minke et al. (1973b), adopted (Minke et al., 1975) and elaborated (Stark and Zitzmann, 1976) to explain the PDA in R1-R6 of Drosophila, used to interpret data from Drosophila phototransduction mutants by Harris and Stark (1976), and assumed to explain PDAs in R1-R6 of Calliphora (Muijser et al., 1975).

In this thesis the phenomenology of the Drosophila PDA in

R1-R6 is examined and compared to that of those in Limulus and Balanus.

4.1: Models of Phototransduction in Drosophila

Questions relating to phototransduction and the mechanism of the afterpotential (PDA) are essentially the same: how does a conformational change in the visual pigment molecule trigger a conductance change at the cell membrane? Despite the widespread and fashionable acceptance of the "excitor-inhibitor" version of the internal transmitter model, convincing independent support has not been forthcoming and at least 3 hierarchical mechanisms of phototransduction remain to be distinguished. The possible links between the isomerisation of the visual pigment and the subsequent conductance change may be conveniently classified as DIRECT, INDIRECT or REMOTE according to the location of the site of conductance change with respect to the visual pigment. The visual pigment of flies lies in the membranes of the rhabdomere (Langer and Thorell, 1966) and the available evidence suggests that the conductance change also occurs here (leech photoreceptor: Lasansky and Fourtes, 1969). Even given this, transmitters (REMOTE phototransduction) are not precluded (Cone, 1973).

1. The conductance change may be a DIRECT consequence of a conformational state of the visual pigment molecule. The rhodopsin molecule is large and forms a major portion of the cell membrane of photoreceptors. Conformational changes in the opsin portion of the molecule are not restricted to those that are spectrally detectable.

2. Alternatively, conformational changes in the visual pigment molecule may trigger events in other molecules which give rise to the conductance change. In this case phototransducton could be called

INDIRECT: that is, intervening steps are involved but, these are not necessarily remote from the visual pigment molecule.

3. Another possibility is that phototransducton is INDIRECT (via intervening steps), and that a molecular species migrates from the area of the visual pigment molecule to initiate a conductance change at a REMOTE site, that is, transmitters are involved (e.g. for vetebrate rods and cones: Yoshikami and Hagins, 1973; for invertebrate rhabdomeric photoreceptors: Cone, 1973). As a special case of the remote phototransducition model we may include the "excitor-inhibitor" model of Hochstein et al. (1973). Ultimately this model requires the demonstration that phototransduction is indirect and remote and involves two species of transmitter which interact.

Evidence which clearly distinguishes between these possibilities (as opposed to data which is conveniently described by a particular model) has proved elusive. Pak's group (see below) have good evidence for the existence of intervening steps in phototransduction (in Drosophila) but their experiments cannot preclude the possibility that these occur on the visual pigment molecule (Pak and Pinto, 1976). Wright and Cosens (1977)(see chapter 2) have good evidence that a component of the membrane conductance of R1-R6 is temporally correlated with the quantity of M580 present in the receptor membrane (of Drosophila) but their data precludes neither intervening steps nor transmitters. Stark's group (see Stark and Zitzmann, 1976; Stark et al., 1976; Harris and Stark, 1976) believe they have identified genes coding for Drosophila R1-R6 "excitor" and "inhibitor" molecules but their data is open to other interpretations. Meanwhile recent work with bovine rhodopsin incorporated into artificial bi-layer membranes demonstrates a DIRECT effect of conformational changes in

bovine rhodopsin upon membrane conductance (Montal et al., 1977)

4.2: Biochemical Evidence of Intervening Steps in Phototransduction in R1-R6 of Drosophila

It should be clear from what was said above that the existence of intervening steps in phototransduction need not imply the existence of transmitters. There is currently no convincing evidence for the existence of transmitters in R1-R6 of Drosophila but the evidence for the existence of intervening steps is compelling.

The voltage response and conductance change associated with the stimulus-coincident response and afterpotential of R1-R6 can be blocked by administration of carbon dioxide or nitrogen. Under these conditions the spectrally detectable visual pigment transitions are known to be normal (Wong et al., 1976). Interestingly, although the electrogenic response is blocked by anoxia, removal of anoxic conditions leaves the photoreceptors in a conductance and voltage state appropriate to their prior illumination (for the afterpotential). This evidence demonstrates an oxygen requiring intervening step in the genesis of the afterpotential. However, once the spectrally detectable visual pigment events have occurred (and perhaps additional unknown events) a signal exists in the photoreceptor which, when oxygen is available, is translated into a conductance change. There is no evidence that this signal is not on the visual pigment molecule and it may reasonably be called a "metarhodopsin signal": it is effectively this, if not in fact. Further evidence for intervening steps in phototransduction in R1-R6 of Drosophila comes from a temperature-sensitive allele of the norpA gene.

The mutant norpA is a phototransduction mutant which has a normal resting potential and resistance, but unlike the wildtype, neither membrane resistance nor potential change when the eye is illuminated (Alawi and Pak, 1971; Alawi et al., 1973). Using a temperature-sensitive allele, norpA-H52 (isolated by M. Heisenberg) Pak et al. (1976) have shown that the visual pigment absorbance change induced by blue light is essentially temperature-independent and the same as that of the wildtype. That is, the spectrally detectable visual pigment transitions appear to be normal. In this mutant, however, in which the receptor potential is normal at 17° C, the receptor potential is progressively degraded at higher temperatures until it is no longer visible at 34°C. Pak et al. interpret this finding as indicating that the norpA gene codes for a protein which intervenes between isomerisation of the visual pigment and the membrane conductance change. That the mutant alleles reflect protein changes is inferred from the fact that a temperature sensitive allele has been found. It is assumed that the protein product of the allele norpA-H52 has a minor conformational error which leads to a breakdown of structure above 34°C (Pak et al., 1976).

Neither study (Wong et al., 1976; Pak et al., 1976) presents evidence which precludes the possibility that the clearly identified "intervening step" is actually a change in the visual pigment molecule which cannot be detected spectrally. Wong's study clearly demonstrates a "latch" effect: the M580 state of the visual pigment (or some unidentified subsequent event) contains a signal which is translated into a conductance change when conditions are favourable (for example when oxygen is available). This result is difficult to reconcile with the "excitor-inhibitor" model but provides strong support for the model of an M580-correlated conductance advanced by

Wright and Cosens (1977)(see chapter 2).

4.3: Suppression of Light Induced Degeneration in rdgB Drosophila: genetic evidence identifying "excitors" and "inhibitors" in R1-R6 of Drosophila?

Apart from the very existence of an afterpotential in R1-R6 of Drosophila none of the phenomenological evidence used by Hochstein et al. (1973) to construct their "excitor-inhibitor" model of phototransduction in Balanus photoreceptors has been reported for the Drosophila photoreceptors. Such evidence is sought in this thesis (chapters 2 and 4). Yet despite the lack of data, Stark's group have forcefully advanced an elaborately modified version of the "excitor-inhibitor" model to explain results obtained from vitamin A deprived Drosophila (Stark and Zitzmann, 1976) and from mutants norpA and rdgB (Harris and Stark, 1977; Stark et al., 1976).

Stark and Zitzmann (1976) describe tha absence of a prolonged blue-light induced afterpotential in the ERG of Drosophila estimated to have about 0.5% of the normal quantity of visual pigment. From this evidence the authors deduce that vitamin A deprivation (1) "inactivates" the afterpotential mechanism, (2) demonstrates the separation of pigment molecules from membrane conductance sites and (3) isolates transduction and adaptation mechanisms. The logic behind these inferences is not made clear by the authors but they are, nevertheless, used to construct an elaborate variation upon the "excitor-inhibitor" theme. Support for the group's model, albeit in slightly modified form, is offered by Harris and Stark (1977).

The Harris-Stark version of the model states the following.

1. A rhodopsin to metarhodopsin transition releases or activates the gene product of the norpA+ gene. This protein is an internal transmitter which acts directly upon the cell membrane by opening sodium channels or "indirectly" by releasing or activating a store of secondary transmitter which reacts with the plasma membrane. Thus the gene product of the norpA+ gene is an "excitor".

2. "Circulating gene product of the rdgB+ gene terminates the action" of the "excitor" by direct interaction with it. The gene product of the rdgB+ gene is an "inhibitor".

The evidence cited by Harris and Stark (1977) in support of this model is the following.

1. In norpA mutants the receptor potential is absent.

2. In rdgB mutants degeneration of R1-R6 occurs after prolonged exposure to light, or irreversibly after exposure to a quantity of blue light which produces a maximal afterpotential in non-mutant flies.

3. Degeneration in rdgB cells R1-R6 is prevented by norpA mutations including an allele which does not block the receptor potential.

Harris and Stark suppose that degeneration occurs in rdgB flies because the "excitor" is not inactivated. Their ad hoc model, however, would not seem to be the only possible explanation of their reported results. Furthermore the model has flaws which are apparent even using their own data. They suggest that the "inhibitor" is

circulating rather than released by M to R transitions - a necessary assertion if the model is to explain their degeneration results - but if this is so the suppression of the afterpotential by orange light (Cosens and Briscoe, 1972) remains unexplained. The suppression of degeneration in rdgB mutants by the presence of the norpA alleles is interesting and the evidence is convincing, but the data provides no evidence upon which to build the model which they have advanced. The data does not even provide evidence for the existence of transmitters.

Any model of phototransduction which claims that the mechanism is indirect requires that the visual pigment transitions have no direct effect upon membrane conductance. Recent work has demonstrated that bovine rhodopsin directly induces a 10 angstrom diameter conductance channel in artificial bilayer membranes after exposure to light (Montal et al., 1977). Free opsin also induces channels which result in discrete conductance fluctuations which would produce discrete voltage fluctuations similar to the "quantum bumps" of invertebrate photoreceptors. Montal et al. (1977) believe that metarhodopsin II in the vertebrate visual pigment cycle may be the first state of the rhodopsin molecule to induce a conductance channel. If invertebrate visual pigment photoproducts were similarly shown to induce conductance channels, the transmitter model of phototransduction in invertebrate photoreceptors (e.g. Cone, 1973) will be seriously challenged, and the data which has so far been interpreted in terms of such models (e.g. the "excitor-inhibitor" model) will need to be re-assessed.

In this thesis an attempt is made to model the afterpotential mechanism using a minimum parameter biophysical model. The result is

an alternative to the "excitor-inhibitor" model (chapter 2) which proves to be consistent with the lastest findings of Montal et al. (1977) and Wong et al (1976).

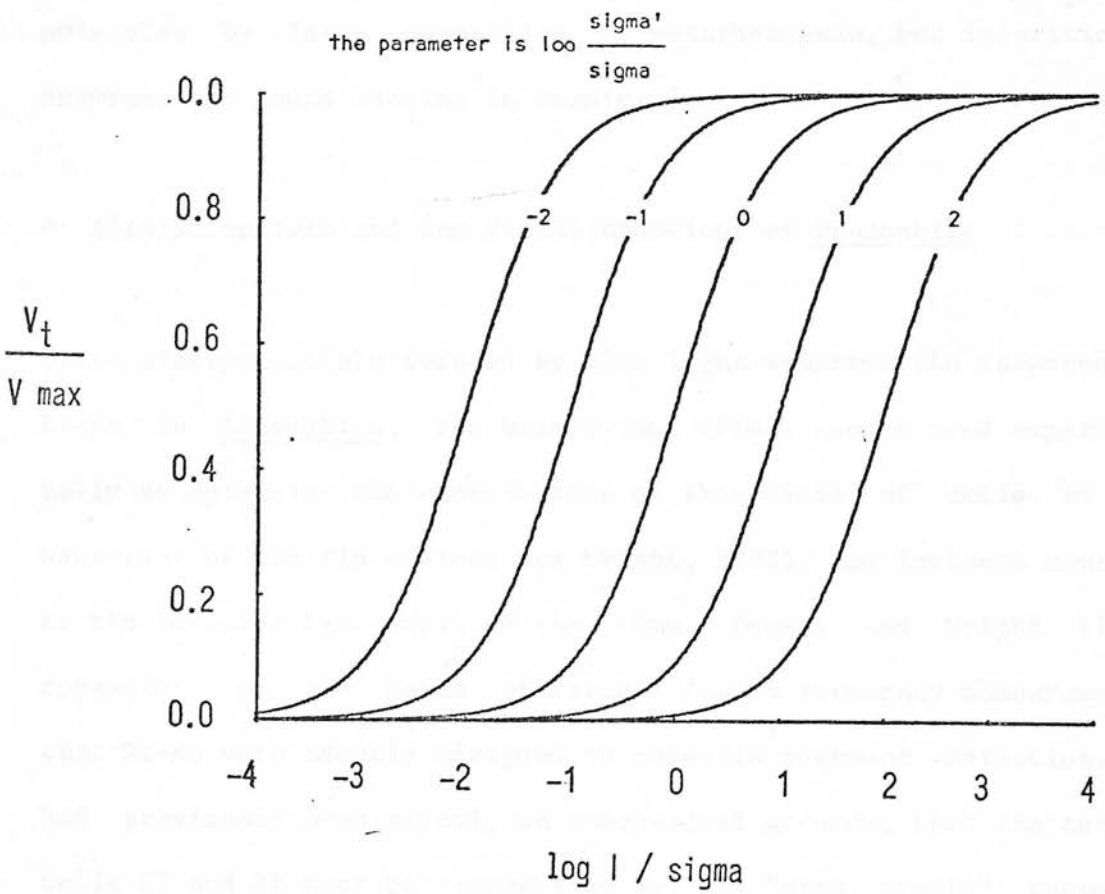
5: Afterpotentials and Receptor Function

Quite aside from the relevance of afterpotentials to the problem of phototransduction, the consequences of afterpotentials for receptor function and for the visual behaviour of the fly are of considerable interest.

Hamdorf and co-workers (Hamdorf et al., 1973; Hamdorf and Rosner, 1973; Rosner, 1975; Hamdorf and Schwemer, 1975) have been pioneers in the study of photoreceptors which contain bi-stable visual pigments. A major interest has been the relationship between rhodopsin concentration and "sensitivity". Rosner (1975, cited in Hamdorf and Schwemer, 1975) manipulated the rhodopsin concentration in the Calliphora retina by spectral adaptation and measured "sensitivity" which was defined as the inverse of the quantity of light required to elicit a criterion voltage response. Rosner concluded that the only effect of an alteration in the rhodopsin to metarhodopsin ratio in Calliphora photoreceptors was to shift the receptor V-log I curve along the horizontal axis (see Fig. 4 for theoretical curves). Further he reports that, contrary to findings in vertebrate photoreceptors (Rodiek, 1973), sensitivity is directly proportional to the concentration of rhodopsin present. Hamdorf and Schwemer (1975) reviewing this work state:

"..... it follows that each light quantum absorbed by the visual pigment contributes the same amount to the receptor potential

Figure 4: According to Hamdorf and Schwemer (1975) a change in the rhodopsin concentration in R1-R6 of Calliphora produces a horizontal shift of the basic sigmoid curve giving parallel curves as shown in this figure. The figure is plotted from calculations using equations similar to those of Lipetz (1969, 1971). If Hamdorf and Schwemer's hypothesis is correct their change in "sensitivity" will correspond exactly to a change in the parameter sigma in the mathematical formulation. See chapters 2 and 3 for details of the equations and a test of Hamdorf and Schwemer's hypothesis as modified by Razmjoo and Hamdorf (1976).



independent of metarhodopsin concentration. In other words the metarhodopsin does not influence the signal transduction....."

In this thesis the Drosophila photoreceptors are examined to determine whether, in Drosophila also, metarhodopsin has no influence on signal transduction. A radically different result holds in Drosophila and the afterpotential associated with metarhodopsin is found to profoundly alter the behaviour of R1-R6.

Razmjoo and Hamdorf (1976) have independently found discrepancies in Hamdorf and Schwemer's hypothesis. They found that "sensitivity" in Calliphora is, indeed, influenced by metarhodopsin. They posit an intermolecular metarhodopsin to rhodopsin interaction that occurs when large quantities of metarhodopsin are present. Their data appears to convincingly demonstrate such a suppression of rhodopsin molecules by large quantities of metarhodopsin, but is critically examined and found wanting in chapter 3.

6: Afterpotentials and the Visual Behaviour of Drosophila

Since afterpotentials induced by blue light suppress the response of R1-R6 in Drosophila, the blue/orange effect can be used experimentally to determine the contribution of this class of cells to the behaviour of the fly (Cosens and Wright, 1975). For instance contrary to the accepted hypothesis of the time, Cosens and Wright (1975) suggested, on the basis of flicker fusion frequency measurements, that R1-R6 were ideally designed to subserve movement detection. It had previously been argued, on theoretical grounds, that the central cells R7 and R8 must be responsible for all "high acuity" responses of the fly because the receptive field of these cells is theoreti-

cally smaller than that of R1-R6 (argument based on rhabdomere diameters: Kirschfeld, 1969). Heisenberg and Buchner (1977) have since, but quite independently, examined a series of Drosophila movement detection responses previously believed to be driven by R7 and R8. Using both receptor cell mutants and the blue/orange effect they isolated the response of the central cells and demonstrated that movement detection ("high acuity") tasks are in fact driven by R1-R6.

All of the physological, visual pigment, and behavioural work relating to the afterpotentials in the Drosophila retina (including that of Heisenberg and Buchner, 1977) has been performed on white-eyed flies. Attempts to induce afterpotentials in the ERG of wildtype Drosophila have been unsuccessful (Cosens and Briscoe, 1972), and no reports have appeared from the groups using intracellular recording techniques. This result leaves open the possibility that the blue/orange effects are artefacts of the various white mutations. This hypothesis is examined in chapter 5 and refuted. Whole field illumination of wildtype Drosophila is found to result in the afterpotential as in the white-eyed fly. This result forms the basis for the interpretation of long-term behavioural modification in wildtype Drosophila reported by Willmund and Fischbach (1977).

The technique of whole field illumination described in chapter 5 has been adapted by Morton (1977) to demonstrate the contribution of R1-R6 to visual fixation reactions in wildtype, cinnabar and white-eyed Drosophila.

7: Aims

The work reported in this thesis was undertaken with the following aims.

To obtain d.c. recordings from the retina of Drosophila in order to describe the behaviour of the ERG afterpotential.

To determine whether the ERG data can provide reliable information about events occurring across the membranes of classes of cells in the retina.

To determine the significance of changes in d.c. potential of the ERG.

To compare the Drosophila afterpotential with that in other species.

To assess models of phototransduction and chromatic adaptation in the light of the collected Drosophila data.

To construct a biophysical model of the Drosophila afterpotential.

To incorporate afterpotentials into mathematical descriptions of sensory receptors.

To determine whether the afterpotential occurs in wildtype Drosophila

To determine whether afterpotentials contribute to behavioural modification in wildtype Drosophila (Willmund and Fischbach, 1977).

CHAPTER 2

BLUE-ADAPTATION AND ORANGE-ADAPTATION IN WHITE-EYED *DROSOPHILA*: EVIDENCE INDICATING THAT THE PROLONGED AFTERPOTENTIAL IS CORRELATED WITH THE AMOUNT OF METARHODOPSIN (M580) IN RETINULA CELLS R1-R6

SUMMARY

1. Short-wavelength light produces prolonged changes in the ERG afterpotential and the size of the ERG response to a fixed intensity testflash: these changes can be reversed at any time by long-wavelength light: this "blue/orange adaptation" is clearly separable from other components of light or dark adaptation (Fig. 3), and operates over a totally different time scale (Figs. 10a and 10b).

2. The amount of light required to produce a criterion change in the state of blue/orange adapation of the eye is independent of the intensity or temporal pattern of the stimulus (Figs. 1, 2, 3, 4, 5, 6, and Table 2).

3. The recovery of sensitivity in darkness following an intense blue stimulus is extremely slow (hours); and at any time during the recovery of sensitivity, blue light will again diminish sensitivity or orange light restore it (Figs. 10a and 10b).

4. The response amplitude elicited by a testflash is highly negatively correlated with the size of the ERG afterpotential (a) during blue-adaptation (Figs. 1, 2, 3), (b) during the decline of the ERG afterpotential resulting from exposure of the blue-adapted eye to long-wavelength light (Figs. 4, 5, 6, 7), (c) during dark recovery from blue-adaptation.

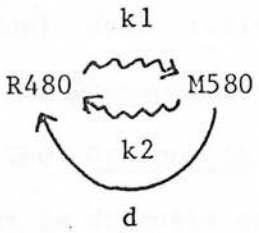
5. The data are consistent with the view that the adaptational

changes described result from interconversions of rhodopsin (R480) and metarhodopsin (M580); that the size of the prolonged ERG afterpotential is closely linked to the quantity of M580 in retinula cells R1-R6 following transduction; that the effects observed in the ERG closely reflect events occurring across the transducing membranes of R1-R6; and that there is a component of the membrane conductance of R1-R6 that is correlated with the quantity of M580 present.

1: INTRODUCTION

The ommatidia of the dipteran compound eye contain an unfused rhabdom: the rhabdomeres of individual retinula cells are separate along their full length and are arranged in a typical asymmetric pattern. Pak (1975) and Pak and Pinto (1976) have recently reviewed the relevant anatomy of the fly retina with particular reference to Drosophila. Kirschfeld and Franceschini on examination of the optics of the compound eye of Musca concluded that it contains two anatomically and functionally distinct systems: one system receives input from the central cells R7 and R8, and the second receives input from the peripheral retinula cells R1-R6 (reviewed in Kirschfeld, 1969). This division has been generally accepted as a property of the dipteran compound eye (McCann and Arnett, 1972) and the two systems differ in their spectral sensitivity: R1-R6 are maximally sensitive to green light (470 to 510 nm) with a second peak in the ultraviolet (340 nm to 360 nm), while R7 + R8 are maximally sensitive to blue light (around 460 nm) and also to UV (Musca, Calliphora and Phaenicia: McCann and Arnett, 1972; Drosophila: Minke et al., 1975; Cosens and Wright, 1975; Harris et al., 1976). Using morphological mutants of Drosophila Harris et al. (1976) demonstrated that R7 is exclusively a UV receptor; a single peaked UV receptor (either R7 or R8) has also been reported for Calliphora (Meffert and Smola, 1976).

The major visual pigments of the Drosophila retina were identified by Ostroy et al. (1974) as a rhodopsin R480 and a metarhodopsin M580 which is stable in darkness (time-constant about 6 hours: Pak and Lidington, 1974) suggesting the simple pigment system



where the wavy lines represent photochemical reactions with rate-constants k1 and k2, that are intensity dependent, and the solid line represents the dark-regeneration of R480 with rate-constant d.

Phenomena associated with this pigment system (Pak and Lidington, 1974) have been localised to R1-R6 by intracellular recordings (Minke et al., 1975). A prolonged depolarising afterpotential (PDA) is accompanied by a reduction in the sensitivity of R1-R6 following an intense blue stimulus; the PDA is abolished by orange light. The ERG of Drosophila had already revealed similar phenomena: an intense blue stimulus is followed by a prolonged corneal negative potential (PCNA) which declines during or after an intense orange stimulus; during the PCNA a residual monophasic response is observed in the ERG (Cosens and Briscoe, 1972). The intracellular evidence of a PDA in R1-R6 (Minke et al., 1975), and ERG data from mutants lacking R1-R6 (Harris et al., 1976) confirm the interpretation of the PCNA presented by Cosens and Wright (1975) and Minke et al. (1975): during a PCNA the contribution of R1-R6 to the ERG is reduced, exposing the monophasic response of the central retinula cells. Harris' study demonstrates that R1-R6 contain the pigment system drawn above; R7 contains a similar system with an R370 and an M470; while R8 is ".....a non-adapting blue-receptor with a third type of rhodopsin" (Harris et al., 1976).

This chapter examines the effects of short-wavelength light (termed blue-adaptation) and long-wavelength light (termed orange-a-

daptation) upon retinula cells R1-R6 by observing the ERG of white-eyed Drosophila.

The Drosophila photoreceptors have not yet been examined in a way that is directly comparable with the studies of Balanus and Limulus (Hochstein et al.,1973; Minke et al., 1973b). In particular the duration of the PCNA or the PDA has not been compared with the long time-constant for the recovery of sensitivity following blue-adaptation in this species (ERG data: recovery of sensitivity takes more than 3 hours: Cosens and Briscoe, 1972; ERG and intracellular evidence: PCNA/PDA last several hours: Minke et al., 1975). The times involved here are similar to the reported time-constant for the dark-decay of the Drosophila metarhodopsin, M580, of R1-R6 (greater than 6 hours, data from the M-potential: Pak and Lidington, 1974) which suggests a possible correlation between sensitivity and the availability of rhodopsin, R480. The issue is complicated by Stavenga's (1975) assertion that the time-constant reported by Pak and Lidington is excessively large. On the issue of sensitivity, Hamdorf's group at Bochum has argued for some years that the rhodopsin concentration of fly photoreceptors controls their sensitivity: the metarhodopsin making no contribution at all (reviewed in Hamdorf and Schwemer, 1975). More recently, however, the importance of metarhodopsin appears to have been realised (Razmjoo and Hamdorf, 1976). Razmjoo and Hamdorf present data showing that the metarhodopsin content of R1-R6 in Calliphora alters the sensitivity of these cells. The data presented in this chapter show that (a) the decline of the PCNA in white-eyed Drosophila is extremely slow and accompanies dark changes in the visual pigment composition of R1-R6 (thus confirming Pak and Lidington's long time-constant for the decay of M580) and (b) the size of the afterpotential is correlated with the quantity of

M580 in retinula cells R1-R6 at any time. It is also shown that (c) the magnitude of the ERG afterpotential and response amplitude are highly negatively correlated in the manner predicted for transmembrane potentials using a model of the transducing membrane. This and other evidence indicates that (d) both ERG and intracellular data concerned with blue/orange adaptation in R1-R6 of Drosophila reflect transmembrane events. Points a, b, and d suggest that (e) the Drosophila data is compatible with an "excitor-inhibitor" model of transduction only if the lifetime of the "excitor" is greater than the lifetime of the metarhodopsin M580. This would mean that the "excitor" had a lifetime of several hours. I suggest the possibility that (f) the quantity of metarhodopsin remaining after transduction determines (via a long-lived "excitor"?) the magnitude of the transmembrane afterpotential which in turn alters the size of the measured response amplitude to a fixed stimulus (and by inference sensitivity) independently of sensitivity changes which may be attributable to the simultaneous changes in the quantity of rhodopsin R480 which is available for photon capture. That is, sensitivity is negatively correlated with the concentration of metarhodopsin M580 in the membranes of retinula cells R1-R6.

2: MATERIALS AND METHODS

2.1: The Flies Used and the Recording Technique

The materials and the ERG recording technique used were similar to those previously reported from this laboratory (Cosens, 1971; Cosens and Wright, 1975). The flies were white eyed (w or bw/cn) Drosophila melanogaster which unlike the red-eyed wildtype, are easily blue/o-

range adapted (Cosens and Briscoe, 1972). They were cultured at room temperature (ca. 22 °C) on standard cornmeal-agar-molasses medium. Electroretinograms (ERGs) from intact flies were monitored on a Telequipment D53 oscilloscope and a Bryans 28000 penwriter via an extracellular Ringer, or sodium chloride, filled glass micropipette. The micropipette was inserted through the cornea into the retinula cell region (peripheral retina) of the eye and led off, via an Ag/AgCl electrode, to a high impedance buffer amplifier. A gold wire inserted into the thorax served as the indifferent electrode.

2.2: Light Sources

The preparation was bathed with light using a collimated beam from a 100 W 12 V tungsten-halogen projector lamp (Philips A1/215) which is called the adapting light. In addition a dimmer light, the testflash, was focussed onto the eye from an overdriven 2.5 V, 0.3 A, torch bulb. This was used for presenting repetitive stimuli and its timing was controlled by a multivibrator with two fixed periods: 2 s and 4 s. The duration of the on-time was continuously variable from a minimum of 180 ms to almost the whole timing period.

With both light sources the wavelength of irradiation was controlled by Balzers broad-band spectral filters. Table 1 shows the transmission characteristics (bandwidth to 10% transmission) of these filters measured with a Pye Unicam SP 800 spectrophotometer. The maximum energy emitted through each filter was measured for both sources using a Tektronics J 16 Digital Photometer and a J6502 probe calibrated in $mW\ m^{-2}$. Measurement of this variable is subject to some error particularly in the case of the testflash, the focussed beam of which does not fill the field of view of the probe. Correction for

Table 1. Characteristics of the filters and light sources used in the experiments.

Filter colour	10% bandwidth (nm)	Adapting light intensity 10^{16} photons $cm^{-2}s^{-1}$	Testflash intensity 10^{15} photons $cm^{-2}s^{-1}$
violet	316-425	0.2	0.8
blue	416-500	1.4	0.8
green	466-546	1.6	1.4
yellow	520-600	3.6	6.3
orange	563-644	2.2	6.2
red	606-670	2.3	6.5
deep red	670-740	1.8	5.9

this is included in the measurements listed in the table. Photon flux was calculated using the relationship: $E = hc/\lambda$, using the midband wavelength of each filter. The intensity of illumination was controlled by calibrated heat-fogged photographic plates or Balzers neutral density filters. A Balzers Calflex BlKl interference heat filter was used at all times.

2.3: Experimental Procedure

Three illumination regimes were used in the experiments reported: (A) Using the adapting light controlled by a photographic leaf shutter: Type 1. Prolonged continuous illumination. Type 2. Brief pulses of light separated, or followed, by periods of darkness. (B) Using the testflash: Type 3. Repetitive alternation of illumination and darkness.

It is a standard aim when testing the response of a receptor to avoid the possibility that the test stimulus alters the performance of the receptor, this involves the use of brief dim stimuli. In a number of the experiments reported I have deliberately used bright test stimuli which do adapt the receptor. This has allowed me to monitor the receptor response continuously during the resultant adaptation. When the testflash has been used in this way it is called the adapting-flash in the text (see Figs. 3, 5, 6, 7). Using an adapting-flash together with a continuous adapting light the receptor response has been observed while it was stimulated by two adapting wavelengths (Figs. 8, 9).

The eyes were selectively adapted using the colour regimes described in Cosens and Wright (1975). These authors, Minke et al. (1975) and Harris et al. (1976) have demonstrated that the phenomena

observed (see Fig. 1) are attributable to retinula cells R1-R6. Thus by observing changes ocurring in the ERG potential during blue and orange adaptation we are principally examining the results of the behaviour of this cell group.

Two basic measurements have been made from the ERG records. The first is the conventional measure of the response amplitude elicited by a fixed intensity stimulus and is an indicator of sensitivity. The second is a measurement of the ERG baseline relative to an arbitrary potential level in the eye; the term afterpotential is used to describe this measurement.

I have adopted the convention of plotting corneal negative potentials upwards on the figures, and afterpotentials are recorded as deviations of the ERG baseline in a corneal negative direction from an arbitrary potential in the region of the initial (pre-blue-adaptation) level of this baseline. Exactly how these measurements were made is clarified in Fig. 1 and the accompanying text.

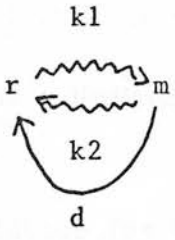
3: RESULTS

3.1: The Kinetics of changes in the Visual Pigment Composition

Different colours of light have been used in the experiments to be reported in order to change the visual pigment composition in R1-R6. This section derives the behaviour of the visual pigment from basic physics. Hamdorf and Schwemer (1975) have given equations which are based on the assumption that the absorption of light in the rhabdomere obeys the Beer-Lambert Law. It is highly unlikely that this assumption is fulfilled in wildtype flies, but it is certainly violated in the white-eyed mutant in which the screening pigments are

absent.

Rhodopsin R480 and metarhodopsin M580 are the only states of the visual pigment in R1-R6 of Drosophila which have been identified (Pak and Lidington, 1974; Ostroy et al., 1974; Harris et al., 1976). The methods used by these workers were insensitive to the existence of transient intermediates of the visual pigment such as have been identified in other species (Limulus: Fein and DeVoe, 1974). Stavenga (1977) has searched for evidence of transient intermediates of the visual pigment in R1-R6 of Calliphora; he found none. If the Calliphora result can be generalised to Drosophila, the visual pigment system of R1-R6 can be represented by



where r and m respectively represent the pigment states R480 and M580, and the diagram is otherwise as given at the beginning of this chapter. The kinetic behaviour of this system (see e.g. Halliday and Resnick, 1966, a basic physics text) is obtained from the following equations

$d = Amr$ $\qquad -dNm/dt = Amr.Nm$

$k1 = Brm.p(v\lambda)$ $\qquad -dNr/dt = Brm.p(v\lambda).Nr$

$k2 = Bmr.p(v\lambda)$ $\qquad -dNm/dt = Bmr.p(v\lambda).Nm$

where N is the number of molecules in the state r or m, B is the Einstein transition probability for absorption for the reaction r to m or m to r at wavelength λ, Amr is the Einstein transition probability for spontaneous emission of m to r (r never decays to m), and $p(v\lambda)$ is the radiation density at wavelength λ. k1 and k2 are seen to be directly proportional to the radiation density within the rhabdomere. The steady state solution of these equations gives

$$\hat{Nr}/\hat{Nm} = (k2 + d)/k1$$

and

$$\hat{nr} = (k2 + d)/(k1 + k2 + d) \qquad \text{(eqn. 3.1-1)}$$

where nr = Nr/Nmax, and Nmax = Nr + Nm

The solution for the kinetic behaviour is

$$nr = \hat{nr} + (nr0 - \hat{nr})\, e^{-Kt} \qquad \text{(eqn. 3.1-2)}$$

where K = k1 + k2 + d

Thus the displacement of the value nr from its equilibrium value $\hat{nr}$ decays exponentially with the time-constant T = 1/(k1 + k2 + d).

3.2: Blue-Adaptation and Orange-Adaptation in the White-eyed Mutant of Drosophila

This section describes the effects of an intense blue light stimulus upon the ERG of a "dark-adapted" Drosophila eye, and the reversal of

these effects following an orange light stimulus.

The time constant (T: time required for 50% change) for the decline of the ERG baseline (afterpotential) following any dose of a long-wavelength stimulus, or a small dose of a short—wavelength stimulus, rarely exceeds 3 s in a fresh preparation. The recovery of sensitivity can be somewhat slower, particularly following an intense stimulus of long duration: T = 10 s to 30 s. These time-constants can be vastly increased following a large dose of short-wavelength light. I will call this effect, and any other effects specific to short-wavelength light in the blue region of the spectrum (i.e. excluding ultraviolet): blue-adaptation. Fig. 1, a penwriter record, illustrates some of the features of blue-adaptation. A brief dim, white light was flashing throughout this demonstration experiment to indicate changes in the response of the eye to a constant stimulus (i.e. to indicate sensitivity changes). Following an intense blue stimulus delivered at "A" on the figure a prolonged corneal negative afterpotential (PCNA) is seen, accompanied by a considerable reduction in the size of the response to the testflash. This response is now monophasic (Cosens and Briscoe, 1972; the "superimposed response", "SR" of Minke et al., 1975) and lacks the lamina on-transient which was previously visible. A point to note is that in all of the figures the ERG data is plotted corneal-negative upwards, this convention facilitates the comparison of extracellular and intracellular recordings.

The slow decline of the afterpotential in the blue-adapted eye may be contrasted with the rapid decline of the afterpotential, and the recovery of the response to the testflash, following intense orange illumination delivered at B on the record. In the remainder of the thesis I will call the adaptation specific to long-wavelength

Figure 1: A penwriter record of the ERG of white-eyed Drosophila demonstrating A blue-adaptation, B the reversal of the effects of blue-adaptation with orange light, and C that blue-adaptation is cumulative when a reduced intensity of blue light is used; the experiment is described fully in the text. At the left hand side of the figure the measurement of the ERG baseline (afterpotential: a) from an arbitrary potential level, and the response amplitude elicited by a testflash (ra) are demonstrated. (From an original recording by D. Cosens).

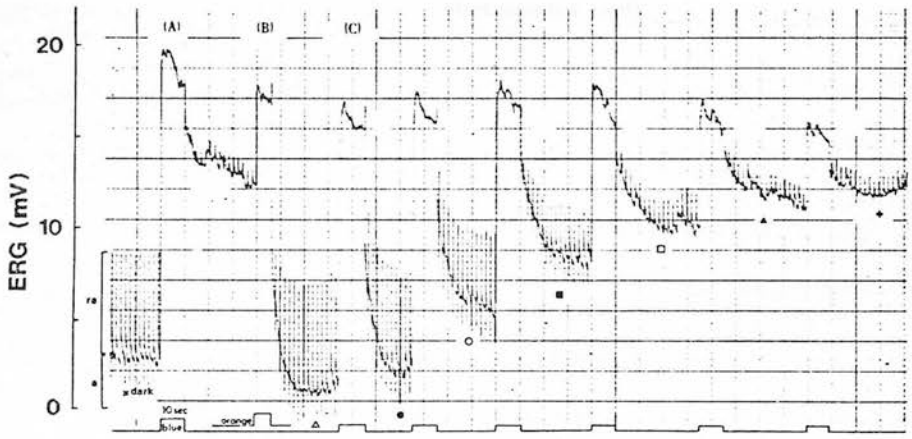
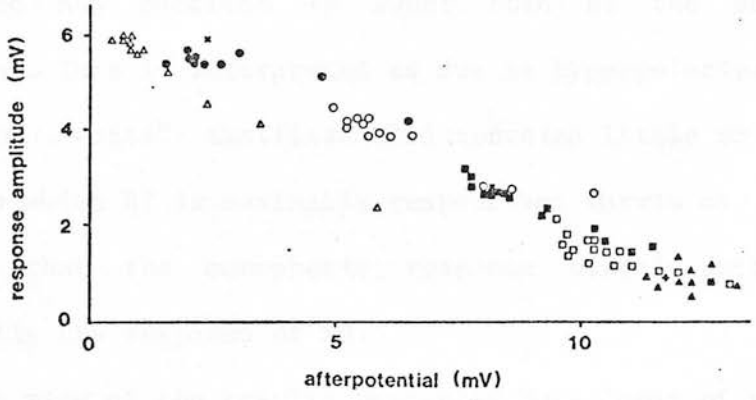


Figure 2: A graph of the response amplitude to the testflash against the afterpotential during the experiments shown in Figure 1. The measurements were taken directly from Figure 1 and the symbols, which are common to Figures 1 and 2, indicate the source of the data.



light in the orange region of the spectrum: orange-adaptation.

The references cited in chapter 1 show that during the PCNA in a blue-adapted eye R1-R6 are depolarised and unresponsive while R7 and R8 are unaffected.

Note that after the orange illumination in Fig. 1 (marked "B"), which converts metarhodopsin back to rhodopsin and removes the PCNA, the ERG baseline is lower than at the beginning of the experiment. This is interpreted as due to hyperpolarisation of R1-R6. Since the "white" testflash used contains little or no ultraviolet light (to which R7 is maximally responsive: Harris et al., 1976) I conclude that the monophasic response visible during the PCNA is principally the response of R8.

In view of the results presented here (most of which have been reported previously by a number of authors: Cosens and Briscoe, 1972; Cosens and Wright, 1975; Minke et al., 1975; Stark et al., 1975; and Harris et al., 1976) it is clearly important to be aware at all times of the state of spectral adaptation of the eye. The remainder of the results in this chapter are concerned with the properties of blue and orange adaptation. Prior to each of the experiments the eyes were dosed with large amounts of either long or short wavelength light (pre-adaptation) which were considered sufficient to make the eye either orange or blue adapted.

The next section examines blue-adaptation with particular reference to the effect of the quantity of blue light delivered in the stimulus; prior to all of the experiments in this section the eye was orange adapted with a large dose of long-wavelength light.

3.3: Blue-Adaptation

Part C of Fig. 1 shows the ERG response when successive doses of blue light of a lower intensity are delivered to the eye. The effect of blue irradiation is cumulative upon (a) the time-constant of decay of the afterpotential (for details see chapter 4), and hence upon (b) the size of the remaining afterpotential measured after an arbitrary fixed time interval, and also upon (c) the size of the ERG response to the testflash. The size of the afterpotential and the response amplitude are highly negatively correlated. Fig. 2 is a graph of measurements illustrating this relationship ($r = -0.98$, $df = 63$, $P<0.01$ for the data from part C of Fig. 1). The source of the points on the graph is indicated by the symbols, which are common to Figures 1 and 2. The symbol "X" shows the mean value of measurements of the afterpotential and the response amplitude at the start of the experiment when the eye was dark-adapted; the symbol "+" shows the mean value of the same measurements after the sixth dose of blue light when the eye was blue-adapted and showing a saturating PCNA.

A correlation between ERG afterpotential and response amplitude appears to be a consistent property of the ERG response in preparations which display d.c. stability and will be observed repeatedly in the remaining results.

The slope of the relationship is not, however invariably the same in a particular preparation using the same testflash. For instance the slope of the relationship is different after the orange illumination shown in Fig. 1 (compare open triangles with the other symbols in Fig. 2). In this case the change in slope occurs immediately after orange light has converted metarhodopsin back to rhodopsin and removed a PCNA, in addition the ERG shows evidence of hyperpolarisation of R1-R6. This result is discussed in chapter 4.

The dynamics of blue-adaptation are clearly separable from

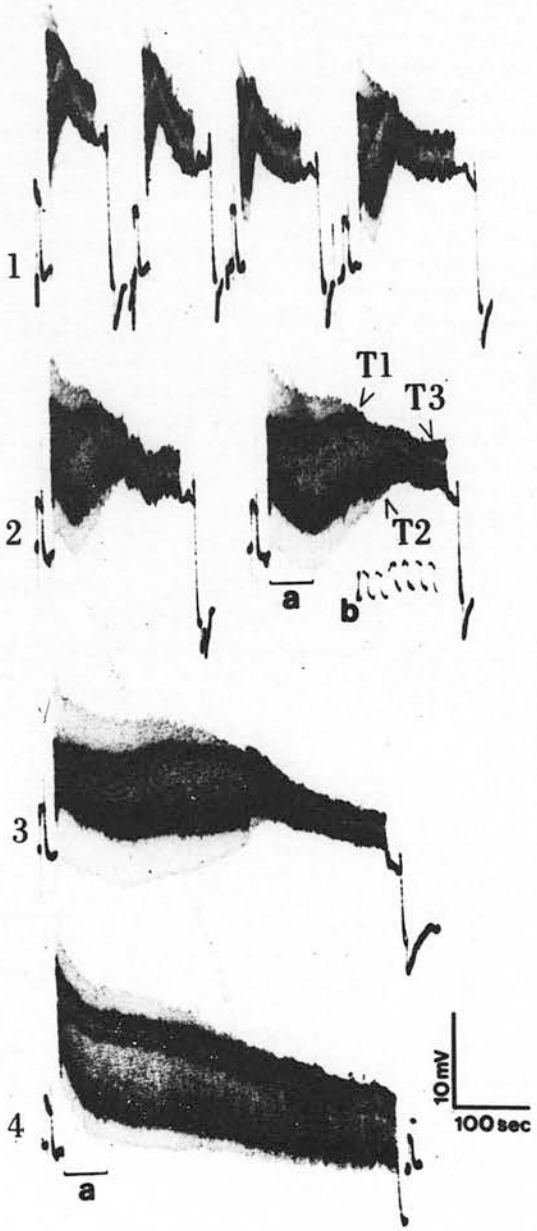
those of spectrally non-specific light adaptation: Figure 3, again penwriter records, illustrates this point. The bottom record (row 4 of Fig. 3) shows, in sequence the ERG response to (a) an intense 10 s stimulus of red light (which orange-adapts the eye) followed by 15 s of darkness, (b) a bright white adapting-flash (1 s on: 1 s off) which continued for several minutes, and was followed by 30 s of darkness, (c) a second 10 s intense red stimulus, followed by a brief period of darkness after which the record ends.

Note that in the record of row 4 the penwriter d.c. offset was used to shift the baseline upwards before the second red stimulus: this shift was not biological. It was necessary to make this shift in this preparation because of the steady downward drift visible on all the records in Figure 3. This kind of drift is probably due to the recording electrodes, but its exact source is not clear.

The broad shaded band forming the major part of each of the records in Figure 3 is the response to the flashing stimulus. The darker central (horizontal) band is the sustained portion of the response attributable to the activity of the retinula cells (top border = the maximum of the corneal negative response due to R1-R8; lower border = the residual afterpotential at the end of the 1 s dark period which separates the flashes). The lighter fringes above and below the dark central band reflect the activity of the lamina. The fringes do not accurately correspond to the lamina transients which are attenuated due to the limited slew-rate of the penwriter. The upper fringe indicates the lamina off-transient, the lower fringe the lamina on-transient.

Returning specifically to the response to a "white" testflash

Figure 3: A montage of ERG records elicited by flashing stimuli. Each record shows, in sequence (a) the ERG reponse to an intense 10 s red stimulus, followed by 15 s of darkness (the eye is now orange-adapted), (b) the response to a flashing stimulus (1 s on: 1 s off), followed by 30 s of darkness (note the PCNA in the records on rows 1, 2 and 3: responses to blue stimuli), and (c) the response to a second 10 s intense red stimulus (which abolishes the PCNAs). Row 4 shows the response when the adapting-flash was white (note that there is no PCNA after the white adapting-flash). The records are interpreted fully in the text. Rows 1, 2 and 3 show responses to blue adapting-flashes of seven intensities (row 1: 7.8, 6.2, 4.9, 1.9; row 2: 1.2, 0.78; row 3: 0.39, X 10^{14} photons cm^{-2} s^{-1}).



(row 4 on Fig. 3), considerable changes in response occur during the first minute or so of illumination (light-adaptation): the lower border of the dark central band (afterpotential remaining after 1 s of darkness) rises and then falls accompanied by negatively correlated changes in the response amplitude (the height of the central band)(see mark A on Fig. 3). As the stimulation proceeds the lamina transients can be seen to decline progressively. The latter of these effects is not true light-adaptation: the lamina transients were restored after either periods of continuous illumination or darkness; the effect is thus a kind of "fatigue" due to the fact that the light is flashing. When the flashing stimulus is terminated the afterpotential declines rapidly; and a red stimulus 30 s later induces no further decline (thus there is no prolonged afterpotential).

Contrast the light-adaptation described in the previous paragraph with the adaptation visible on the remaining records of Figure 3 (rows 1 to 3). The stimulus regime resulting in these records was similar to that described above except that the flashing stimulus was a blue light of a range of intensities (row 1: 7.8, 6.2, 4.2, 1.9; row 2: 1.2, 0.78; row 3: 0.39, X 10^{14} photons cm^{-2} s^{-1}). Again light-adaptation is visible, particularly on the second record of row 2 (marked A), but a completely new component has appeared: blue adaptation. Blue-adaptation is visible in the records of Figure 3 as (a) an asymptotic, or overshooting, rise of the afterpotential (the lower border of the dark central band of the ERG) accompanied by (b) a negatively correlated progressive decline of response amplitude and, in sequence, (c) the gradual elimination of the lamina off-transient (upper fringe) and (d) the lamina on-transient (lower fringe) followed by (e) the appearance of a new corneal-negative on-transient of slower speed, labelled T3 on the figure. This slow corneal

negative on-transient is the spike of the response of the central retinula cells R7 and R8, it consistently becomes more prominent in the ERG when the peripheral retinula cells R1-R6 are blue-adapted.

The afterpotential changes associated with blue-adaptation are prolonged, for instance a PCNA is visible when the blue flashing light is switched off (rows 1 to 3 on Fig. 3). This PCNA declines during or immediately after the second 10 s red stimulus.

The response remaining after blue-adaptation (see inset B) is monophasic. This and the saturating, or near saturating, PCNAs visible during the 30 s dark period following blue-adaptation show that R1-R6 are almost completely unresponsive after the doses of blue light applied. Results presented in chapter 4 show that the PCNA may settle at levels intermediate between the "dark-adapted" and "saturated" levels of the ERG baseline when intermeidiate doses of blue light are delivered to the eye. Under those conditions R1-R6 may remain partially responsive during a PCNA.

Stark (1975) reported that, over a wide range of intensities, the quantity of blue light required to produce a criterion change in ERG response is independent of the intensity of light used. My data are in agreement with his finding: I found a high correlation between the duration of the illuminaton required to produce three different criterion changes in the ERG and the reciprocal of the blue light intensity inducing the adaptation. Correlation coefficients obtained using data from Figure 3 and a replicate with the same preparation are tabulated in Table 2. In the experimental situation used, of the order of 10^{16} photons per square centimetre are required to produce a saturating PCNA.

The results demonstrate that the critical variable determining

Table 2. Inverse correlations between intensity and duration of blue light required to produce three criterion changes in ERG response.

Criterion	Label on Figure 3	r	df	p
loss of lamina off-transient	T1	0.99	12	< 0.01
loss of lamina on-transient	T2	0.99	12	< 0.01
appearance of new on-transient	T3	0.98	10	< 0.01

the extent of blue-adaptation is the quantity of blue light in the stimulus. This implies that the progress of blue-adaptation is related to a simple photochemical conversion creating a product which has a large dark-decay time-constant: presumably the generation of metarhodopsin M580 in R1-R6 which behaves in this way (Harris et al., 1976); the time-constant reported for the dark-decay of M580 is about 6 hours (Pak and Lidington, 1974).

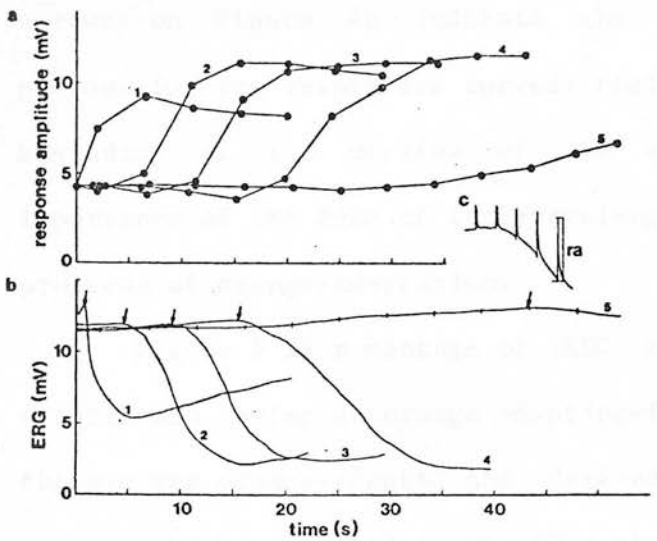
3.4: Orange-Adaptation of Blue-Adapted Eyes

In this section the behaviour of the ERG during orange-adaptation of blue-adapted eyes is reported, it is presumed that during orange-adaptation metarhodopsin M580 is converted back to rhodopsin R480 (e.g. Harris et al., 1976).

The three types of illumination regime described in the methods section were used to orange-adapt blue-adapted eyes. The following key points emerge: (a) in each case the curve of afterpotential decline is sigmoid, (b) the curve of recovery of response amplitude is also sigmoid and follows a similar, but mirror image, time course to the afterpotential decline, (c) the response amplitude to a fixed intensity stimulus is negatively correlated with the size of the afterpotential and (d) progress along the sigmoid curve of afterpotential decline is determined by the dose of orange light delivered in the adapting stimulus (time for 50% decline of afterpotential is inversely proportional to the intensity of illumination used for the adaptation).

Figure 4 shows data from an experiment in which blue-adapted eyes were orange-adapted with continuous red light. The intensity of red light producing the changes visible in curves 1, 2, 3, 4 and 5

Figures 4a and 4b: Changes in the ERG of the blue-adapted eye as it is orange-adapted by a continuous red light; 4a shows measurements of the response amplitude elicited by a white testflash; 4b is drawn from tracings of the ERG baseline: the ordinate is measured relative to the orange-adapted and dark-adapted level of the baseline. The curves thus show the residual afterpotential. The data plotted was obtained using 5 intensities of red light to orange-adapt the blue-adapted eye. The red intensities resulting in curves 1, 2, 3, 4 and 5 were 23.2, 2.32, 1.07, 0.56, 0.23, X 10^{15} photons cm^{-2} s^{-1}. The arrows on Figure 4b indicate that an equal number of photons has been delivered to the preparation when the decline of the afterpotential begins. (From original recordings by D. Cosens).



was 23.2, 2.32, 1.07, 0.56 and 0.23, X 10^{15} photons cm^{-2} s^{-1}, respectively. Figure 4a shows the response amplitude elicited by a constant intensity white testflash (response amplitude measured as shown in inset c) and Figure 4b was compiled from tracings of the ERG baseline, during the red illumination in each experiment. In each case the red illumination caused a decline of the ERG baseline (afterpotential) along a sigmoid curve. The final level of the baseline is equivalent to the stimulus-coincident response from the orange-adapted eye. Curve 5 reached this level in about 90 s. The duration of the adapting illumination required for 50% of the observed decline of the afterpotential is inversely correlated with its intensity ($r = 0.99$, $df = 3$, $P<0.01$) and, as can be seen from the figure, afterpotential and response amplitude follow a similar (but mirror image) curve; and they are thus negatively correlated. The arrows on Figure 4b indicate the delivery of an equal number of photons for the respective curves: their location with respect to the beginning of the decline of the afterpotential illustrates the importance of the dose of long-wavelength light in determining the progress of orange-adaptation.

Figure 5 is a montage of ERG recordings from a series of experiments using an orange adapting-flash. Prior to each experiment the eye was orange-adapted and dark-adapted. The illumination sequence which resulted in the ERGs shown was: (a) orange adapting-flash (1 s on, 1 s off) applied, then switched off, (b) 10 s of intense blue light applied and followed by 30 s of darkness (note the PCNA in the now blue-adapted eye), (c) orange adapting-flash re-applied (inducing the decline of the afterpotential and recovery of response amplitude) and finally switched off. The montage shows records obtained using a series of orange light intensities: row 1:

Figure 5: A montage of ERG recordings illustrating orange-adaptation. Each record shows in sequence, the resposne of the eye to (a) an orange adapting-flash (1 s on: 1 s off), followed by a brief dark period, (b) an intense 10 s blue stimulus, followed by 30 s of darkness (note the PCNA in the now blue-adapted eye), and (c) the re-applied orange adapting-flash. The records were obtained using ten intensities of orange adapting-flash (row 1: 6.2, 4.9, 3.9, 3.1; row 2: 1.49, 0.99, 0.6; row 3: 0.29, 0.15; row 4: 0.05, X 10^{15} photons cm^{-2} s^{-1}).

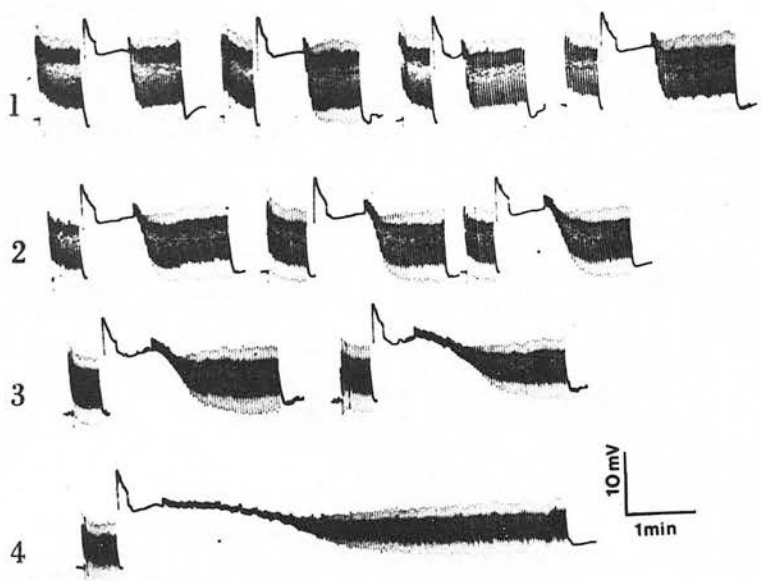


Table 3. Regression slopes and correlation coefficients of response amplitude on afterpotential: data from Fig.5.

testflash intensity 10^{15} photons $cm^{-2}s^{-1}$	slope	r	p <	df = 5 to 25
6.2	-0.81	-0.998	.01	
4.9	-0.68	-0.996	.01	
3.9	-0.74	-0.994	.01	
3.1	-0.65	-0.993	.01	
1.49	-0.63	-0.992	.01	
0.99	-0.55	-0.987	.01	
0.6	-0.51	-0.985	.01	
0.29	-0.51	-0.957	.01	
0.15	-0.45	-0.938	.01	
0.05	-0.45	-0.957	.01	

The slope of the regression approaches -1, and more variance is explained by linear correlation, as It increases-as predicted by the model.

Variance explained = r^2.

6.2, 4.9, 3.9, 3.1; row 2: 1.49, 0.99, 0.6; row 3: 0.29, 0.15; row 4: 0.05, X 10^{15} photons cm^{-2} s^{-1}. The records illustrate again (a) the sigmoid decline of afterpotential and sigmoid recovery of response amplitude, (b) the dependence of the rate of these changes upon the intensity of illumination (time required for 50% of the observed decline in afterpotential is inversely correlated with the orange adapting-flash intensity: $r = 0.994$, $df = 8$, $P<0.01$), and (c) the negative correlation between the size of the afterpotential and the response amplitude. This last correlation becomes increasingly curvilinear as the intensity of the flashes is reduced. A very dim flash is not seen to increase the response amplitude until the ERG afterpotential is substantially reduced (for example see the record in row 4 of Fig. 5). A possible interpretation of this is considered in the discussion. Despite this effect, high linear correlations between afterpotential and response amplitude were obtained from all of the records in Figure 5, with no correlation coefficient worse than $r = -0.95$ ($df = 5$ to 25, $P<0.01$; see Table 3).

Figures 6 and 7 illustrate the key points of interest in this section. Both of the figures are drawn from the same data from experiments of the type shown in Figure 5. Data for two intensities of orange adapting — flash (180 ms on, 1,820 ms off) have been plotted: 6.2 X 10^{15} photons cm^{-2} s^{-1} and one tenth of this. Figure 6 shows the percentage change in the afterpotential (solid symbols) and the percentage change in the response amplitude (open symbols), during the orange-adaptation, plotted against the number of photons delivered to the eye. In relation to the time scale in the original experiments, the time scale for the curve of lower intensiy (triangles) is compressed by a factor of ten compared with that of the higher intensity (circles). Figure 7 is a graph of the response

Figure 6: A graph showing the percentage change in the ERG afterpotential (solid symbols), and the percentage change in the response amplitude to an orange adapting-flash (open symbols), during orange-adaptation against the number of orange photons delivered to a blue-adapted eye. The graph is derived from measurements of records of the type shown in Figure 5. Curves from experiments using two intensities of adapting-flash are defined by the symbols: triangles: 6.2; circles: 0.62, X 10^{15} photons cm^{-2} s^{-1}.

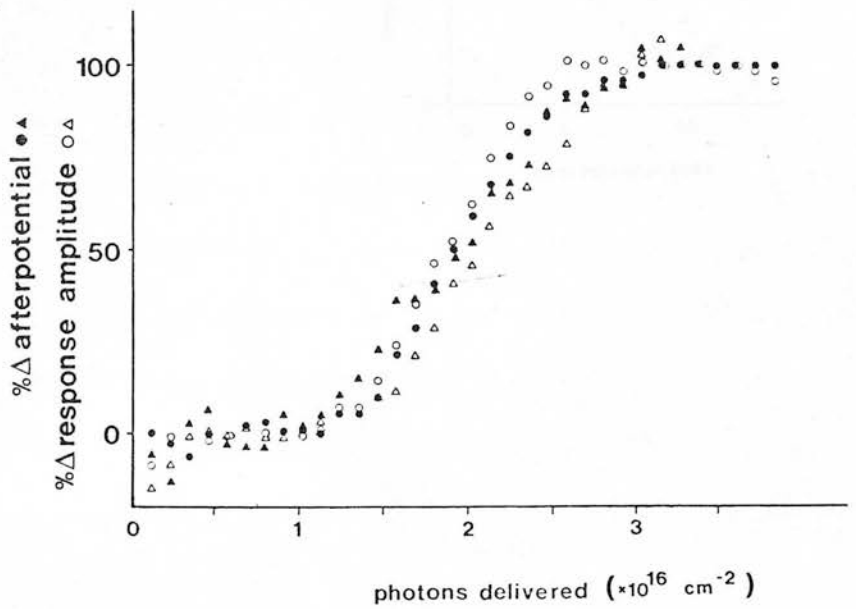
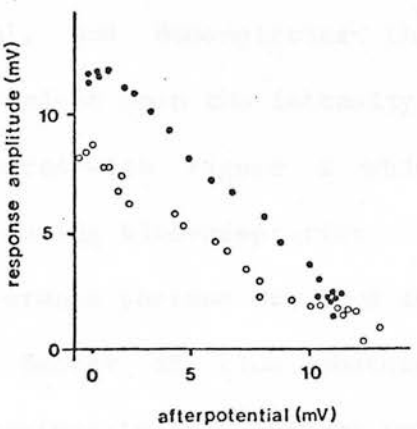


Figure 7: A graph of measurements of the response amplitude against the afterpotential from the same records that were used to draw up Figure 6 (solid circles: 6.2; open circles: 0.62, X 10^{15} orange photons cm^{-2} s^{-1}).



amplitude plotted against the afterpotential (solid circles = higher intensity, open circles = lower intensity). Figure 6 clearly demonstrates that the decline of the afterpotential is closely dependent upon the quantity of orange light delivered to the eye (compare the curves defined by the solid symbols); and that the recovery of the response amplitude closely follows the decline of the afterpotential (compare the like symbols). This close correspondence becomes increasingly distorted as the testflash intensity is reduced. Figure 7 illustrates the negative correlation between the response amplitude and the afterpotential, and demonstrates that the slope of the relationship is dependent upon the intensity of the testflash. This figure should be compared with Figure 2 which shows the similar correlation observed during blue-adaptation.

The number of orange photons required to orange-adapt the eye is similar to the number of blue photons which result in full blue-adaptation (approximately 10^{16} photons cm^{-2}).

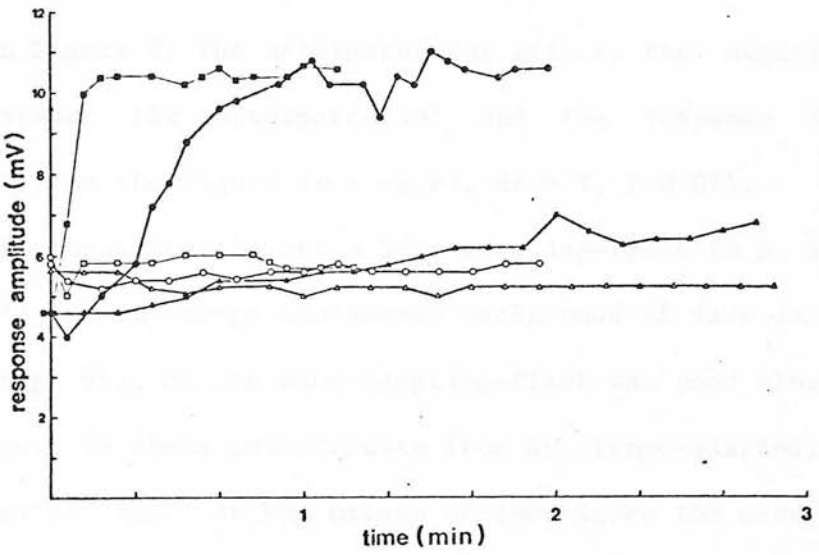
The data so far presented show that the size of the afterpotential during blue and orange adaptation is sigmoidally related to the number of photons (either blue or orange) that have been absorbed by the visual pigment; and suggest that orange — adaptation simply reverses the process of blue-adaptation. It is implied that blue-adaptation is due to the accumulation of the metarhodopsin M580, that orange-adaptation is due to the elimination of M580 and consequent regeneration of rhodopsin R480, and that the prolonged afterpotential is closely related to the composition of the visual pigment. The data further show that while the PCNA is present it is highly negatively correlated with (determines?) the size of the response to a testflash, and hence the sensitivity of R1-R6. This relationship between response amplitude and the afterpotential will be called the "after-

potential effect". It is further investigated in the next section.

3.5: ERG Measurements during Exposure of the Eye to Two Antagonistic Wavelengths of Light

Classically the sensitivity of a photoreceptor is reduced during illumination with a background light. It should be clear from the preceding results (e.g. Fig. 4) that this is not the case in Drosophila if a blue-adapted eye becomes orange-adapted by the background illumination. We saw (e.g. Fig. 3) that a short wavelength adapting-flash induces a PCNA and a reduction in response amplitude to the monophasic response of the central cells R7 and R8; Figure 8 describes the changes in response amplitude which occur when an orange-adapting background is added to such an adapting-flash. The data on the figure begins with the eye blue-adapted at the moment when the orange-adapting background is added. Both lights remained on throughout the experiment and the response amplitude plotted is the response of the eye to the short – wavelength adapting-flash. The on-transient of the monophasic response of the central cells was identifiable throughout these experiments in this preparation, allowing continuous assesment of the contribution of these cells to the ERG response (open symbols). This response barely changes during the experiments and clearly the central cells are barely affected. In contrast, the total response (solid symbols) changes markedly, and the response size increases during the long-wavelength illumination: presumably due to the elimination of M580 and the regeneration of R480 in R1-R6. Curves for three intensities of red background light are shown (squares: 2.3; circles: 0.23; triangles: 0.023, X 10^{16} photons cm^{-2} s^{-1}) and it is clear from the figure that the rate at which the equilibrium value of response amplitude

Figure 8: A graph showing the time-course of the change in the response amplitude elicited by a green adapting-flash (which partially blue-adapts the eye) when a red background is added. The solid symbols show measurements of the total response amplitude while the open symbols show measurements believed to correspond to the monophasic response of the central cells, which was separately measureable in this particular preparation throughout the experiments. Data obtained using three intensities of red background are plotted in the Figure (squares: 2.3; circles: 0.23; triangles: 0.023, X 10^{16} red photons cm^{-2} s^{-1}). (From experiments by D. Cosens).

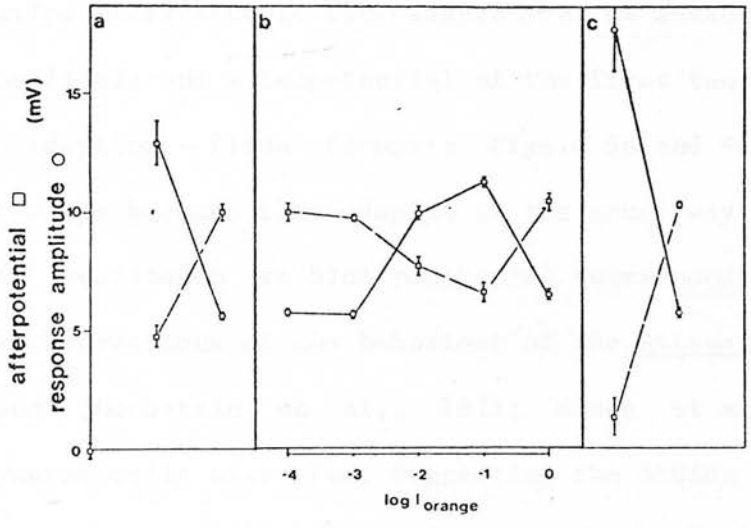


is attained is dependent on the intensity of red illumination: a result which is consistent with the idea that the change in sensitivity is related to a photochemical change.

The results of Figure 8 could readily be interpreted as indicating that the response amplitude from, and hence sensitivity of, R1-R6 is determined entirely by the concentration of rhodopsin R480 present in these cells following photochemical conversion of metarhodopsin M580 by red light. It proves, however, that this cannot be the whole story for, as before, the afterpotential effect is operating. Figure 9 demonstrates this by plotting data (mean + 95% confidence limits, n = 10) on the afterpotential (squares) and the response amplitude (circles) from experiments similar to that described in Figure 8. The afterpotential effect, the negative correlation between the afterpotential and the response amplitude, is apparent from the figure ($r = -0.97$, $df = 7$, $P<0.01$).

In these experiments a blue adapting-flash (8×10^{14} photons cm^{-2} s^{-1}) and an orange continuous background of five intensities was used (Fig. 9b), or the blue adapting-flash was used alone (Figs. 9a, 9c). Figure 9a shows measurements from an orange-adapted, and 10 min dark-adapted eye. At the origin of the figure the mean value of the dark-adapted afterpotential is shown; the response amplitude elicited by the first blue flash was 20.7 mV. Mean measurements of the first ten responses to the blue adapting—flash, and of ten resposes when the response had come to equilibrium (eye blue-adapted) are plotted. Measurements made 1 min after an orange background had been added to the blue adapting-flash illuminating a blue-adapted eye are plotted in Figure 9b. As the intensity of the orange light increases there is an increase in the response amplitude and a reduction in the size of

Figure 9: Afterpotential (measured from the orange-adapted, dark-adapted level of the ERG baseline) and response amplitude elicited by a blue adapting-flash (8 X 10^{14} photons cm^{-2} s^{-1}), 9a of the first 10 responses to the blue adapting-flash and 10 responses when the eye was blue-adapted, 9b 1 min after an orange background light was added to the flashing blue light, and 9c identical to 9a except for the prior adaptation. The data plotted in 9c was obtained from the ERG of the eye immediately after the orange/blue mixing experiment at the maximum orange intensity. Mean values (+ 95% confidence limits, n = 10) of measurements of the afterpotential (squares) and the response amplitude (circles) are plotted in the figure. The orange intensities in part 9b are relative to 2.2 X 10^{16} photons cm^{-2} s^{-1}.



the afterpotential. At the maximum intensity of the orange light the measured afterpotential rises due to the stimulus-coincident corneal negative response of the eye, and the response amplitude elicited by the adapting-flash falls. (This observation is typical of observations of response amplitude and afterpotential when, say, an orange background is added to an orange testflash). The measurements of response amplitude and afterpotential at this intensity fall on the same regression line as the remaining data from Figure 9, which strongly suggests that the afterpotential is a primary determinant of response amplitude and hence of sensitivity.

Figure 9c shows measurements obtained under identical experimental conditions to those of Figure 9a except in respect of the prior adaptation of the eye. Figure 9a shows data from the eye after 10 minutes of darkness following orange-adaptation: Figure 9c shows data obtained from the eye immediately after the blue/orange mixing experiment at the maximum intensity of orange illumination; the eye shows a marked resistance to blue-adaptation, as measured by the mean response amplitude and afterpotential of the first ten responses to the blue adapting – flash (compare Figs. 9a and 9c); eventually, however, the eye becomes blue-adapted in the usual way.

This resistance to blue adaptation corresponds superficially to similar observations of the behaviour of the Balanus and Limulus preparations (Hochstein et al., 1973; Minke et al 1973b) for it decays asymptotically with time, suggesting the action of "inhibitors". However, in Drosophila the resistance is not absolute as appears to be the case in the other species, and determines only the quantity of short-wavelength light which is required to blue-adapt the eye.

3.6: Dark Changes in the ERG of Fully Blue-adapted Eyes

The independence of the dark decline of the PDA from the dark-decay of metarhodopsin, observed in the Balanus and Limulus preparations previously described (Hochstein et al, 1973; Minke et al., 1973b), provided crucial evidence for the "excitor-inhibitor" model of phototransduction in these species. The decline of the PCNA in the fully blue-adapted Drosophila eye is so protracted that it is difficult to make a continuous recording of it before (a) the preparation dies (after several hours) or (b) apparently spurious drifting of potential (either in the fly or in the recording equipment) is greater than the effect we are trying to observe. Dark recovery from blue-adaptation was therefore followed by monitoring the response of the eye to three dim green testflashes at various intervals after blue-adaptation. This method immediately detects problem "a" as it arises, and largely overcomes problem "b"; the afterpotential size can then be assessed at the points where the eye was subsequently orange-adapted.

Figure 10 reports data obtained using experiments of the type described above. Figure 10a shows the time-course of the recovery of the response amplitude elicited by the testflash (as a percentage of the initial, pre-blue, dark-adapted response size) after blue-adaptation (circles) and orange-adaptaton (squares). These measurements were taken from the records of the experiment which is also the source of the data represented by the solid squares on Figure 10b. This figure shows long-term changes in response amplitude after blue-adaptation. In this case the response amplitude is calculated from (ra - rab)/(ra0 - rab), and expressed as a percentage. In this equation, ra = the measured response amplitude; rab = the response amplitude 1 min after blue-adaptation; and ra0 = the initial,

Figure 10a: Short term changes in response amplitude as a percentage of the pre-blue response amplitude, ra0) after blue and orange illumination. Long term changes from the same experiment are represented by squares on Fig. 10b. The squares show response amplitudes measured after the orange illumination oversize squares) on Fig. 10b; The circles show measurements made after blue illumination (oversize circles) on Fig. 10b. The data represented by solid symbols corresponds to the events shown on Fig. 10b; the open symbols show data from events 10 hours from the beginning of this experiment: the similarity of time-courses after long periods in the blue-adapted state is striking.

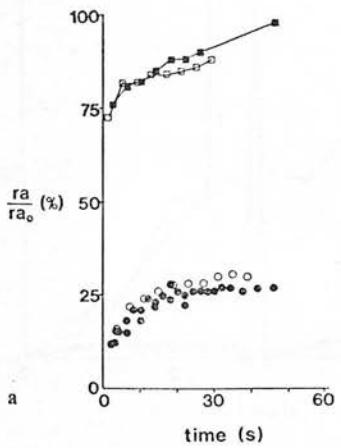
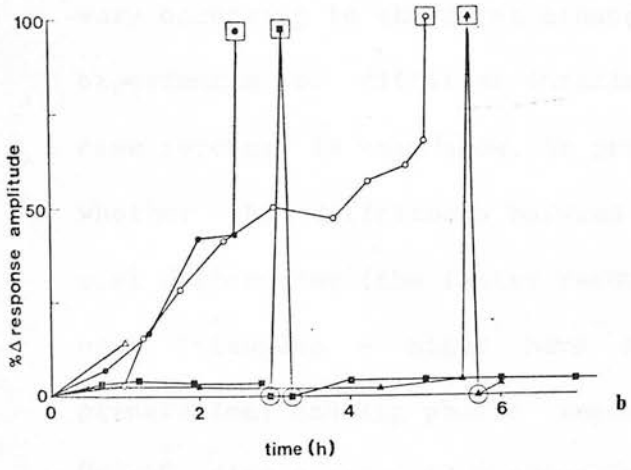


Figure 10b: The percentage change in response amplitude occurring during long periods of darkness following blue-adaptation. The oversize squares show measurements made 1 min after an intense long wavelength stimulus; oversize circles indicate measurements made 1 min after an intense short wavelength stimulus. In calculating the data plotted on this graph the response amplitude measured 1 min after the initial blue-adaptation was subtracted; the recovery of response amplitude plotted on this figure is believed, therefore, to show the recovery of R1-R6 from blue-adaptation.



pre-blue, dark-adapted response amplitude: thus the changes plotted on the figure exclude the recovery during the first minute after blue adaptaton. The early portion of the recovery is believed to be due to dark-adaptation of the central cells, while the subsequent recovery is largely recovery of R1-R6. It is clear from Figure 10a that after blue-adaptation (circles) the recovery of the monophasic response is near its asymptote within 1 min (rab) and that the shape of the recovery curve is similar after each of the blue-adapting stimuli (indicated by oversize circles on Fig. 10b); similarly the recovery of the orange-adapted response (squares) following long — wavelength illumination (indicated by oversize squares on Fig. 10b) is unchanged. The open symbols on Figure 10a plot data obtained 10 hours after the beginning of the experiment.

Figure 10b shows the percentage of recovery of the response amplitude in darkness following blue-adatation, excluding the recovery occurring in the first minute (see Fig. 10a). Data from five experiments of different duration are plotted on the graph; in each case recovery is very slow. At present it is not possible to state whether the differences between the curves are due to (a) experimental differences (the faster recovering preparations - circles and open triangles - might have been due to stray light reaching the preparation, causing photic regeneration of rhodopsin R480), (b) "real" differences in recovery rates between flies, or (c) differences in the amount of metarhodopsin M580 accumulated during the blue-adaptation; if this last is the case, and less M580 was present in the flies producing the faster recovering curves, then it is clear that the recovey of the response amplitude follows a sigmoid time-course. The following points emerge unambiguously from Figure 10b, (a) the recovery of the response amplitude elicited by a fixed

intensity stimulus is extremely slow (hours) and this time-course is of the same magnitude as that reported for the dark-decay of metarhodopsin M580 in Drosophila (6 h: Pak and lidington, 1974), (b) long-wavelength light restores the response size at any stage of dark recovery (indicated by oversize squares on Fig. 10b; see also Fig. 10a), (c) short-wavelength light reduces the response size to the blue-adapted level either after long periods of darkness following blue-adaptation or following orange adaptation (the responses following short-wavelength adapting stimuli are marked by oversize squares on Fig. 10b).

These data indicate a close relationship between the state of blue/orange adaptation of the eye (measured by the response to the testflash) and the visual pigment composition of R1-R6. The size of the afterpotential was assessed at the times when the eye was switched out of the blue-adapted state (oversize squares on Fig. 10b); although the overall size of the ERG response was sometimes reduced after long periods of blue-adaptation, a clear reduction in the size of the afterpotential was observed after the long-wavelength adaptation and the response amplitude and the size of the afterpotential were, again, highly negatively correlated: it is concluded that the PCNA has a duration of several hours and that its magnitude can be predicted from the response amplitude data in Figure 10b.

This finding in white-eyed Drosophila contrasts sharply with the behaviour of the PDA reported in the Balanus and Limulus preparations (Hochstein et al., 1973; Minke et al., 1973b). The data confirms that the decay of Drosophila metarhodopsin M580 (Pak and Lidington, 1974) and the recovery of ERG sensitivity after blue-adaptation (Cosens and Briscoe, 1972) are processes that require several hours, and demonstrate that the decline of the ERG afterpotential

runs in parallel with these processes. Minke et al. (1975) also report that the PCNA and the PDA observed in Drosophila may last several hours. Thus in Drosophila, where both intracellular (PDA: Minke et al., 1975) and extracellular (PCNA: this chapter and Minke et al., 1975) evidence of transmembrane afterpotentials in R1-R6 exists, it is clear that measurements of sensitivity may be biased by the afterpotential effect, even several hours after spectral adaptation.

4: DISCUSSION

The data presented in the results section make it clear that the blue/orange adapting behaviour of R1-R6 in white-eyed Drosophila (judged from the ERG evidence) differs from the spectral adaptation behaviour of Balanus lateral ocelli and Limulus median eye (intracellular data: Hochstein et al., 1973; Minke et al., 1973b). Their are also some differences between the Drosophila data presented and the reported behaviour of R1-R6 in Calliphora, the other fly in which PDAs have been reported (intracellular recordings: Muijser et al., 1975). As in the Balanus and Limulus preparations, certain specific histories of spectral adaptation appear to be pre-requisites if a PDA is to be observed: the specific nature of these histories argues in favour of the "excitor-inhibitor" model of visual excitation applying in Calliphora (Muijser et al., 1975).

I have found no evidence, in Drosophila, of states of the eye from which blue-adaptation will not arise given a sufficient blue stimulus: PCNAs follow a large blue stimulus (a) shortly after orange recovery from blue-adaptation (Figs. 1, 9c, 10a, 10b), (b) shortly after an orange pulse delivered to an orange-adapted eye (Figs. 3 and

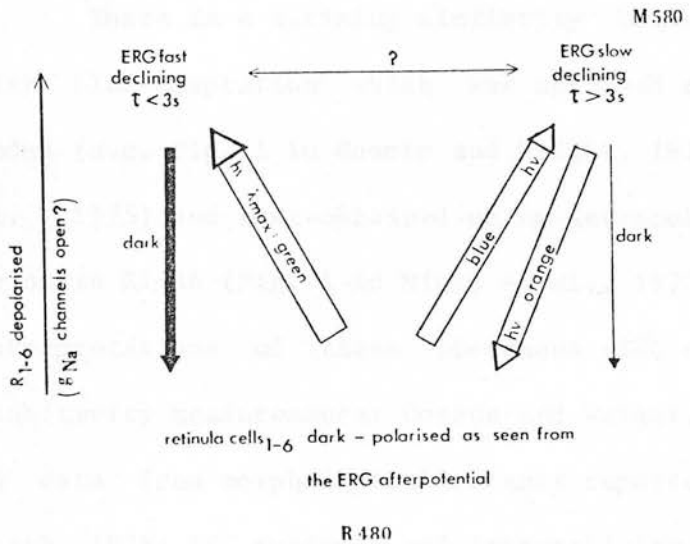
5), (c) after long periods of darkness following blue-adaptation (Figs. 10a and 10b), (d) after partial recovery from partial blue-adaptation (i.e. blue-adaptation is cumulative: Figs. 1, 2 and 3) and (e) 10 min after orange-adaptation (Fig. 9a). There are quantitative differences in the amount of blue light required to produce a criterion change in the ERG (compare Fig. 9a with Fig. 9b). Except in "old" preparations (where all aspects of the behaviour of the ERG are slowed down) I have found no states of the eye in which orange recovery from blue-adaptation is not possible.

While the evidence presented fails to corroborate the hypothesis that an "excitor-inhibitor" interaction underlies visual excitation and the afterpotential mechanism in R1-R6 in Drosophila it is unable to refute the model.

4.1: The Electrophysiological Behaviour of R1-R6 in White-Eyed Drosophila

A schematic description of the basic featues of the electrophysiological behaviour of R1-R6 of Drosophila is given in Figure 11. The description is based on the wavelength- specific effects on the rate of dark-decline of the afterpotential following a stimulus. The ERG fast decling response is that assayed by sensitivity measurements and is maximally sensitive in the green region of the spectrum (wavelength maximum around 490 nm: Cosens and Wright, 1975; Minke et al., 1975). The ERG slow-declining response (PCNA and PDA, i.e. the response associated with blue adaptation) is most sensitive to blue light (wavelength maximum around 460 nm) and is most readily reversed by orange light (wavelength maximum around 580 nm: see Fig. 5 in Stark, 1975).

Figure 11: A schematic description of the electrophysiological response of R1-R6 in white-eyed Drosophila. See text for discussion.



A much greater number of photons is required for complete blue-adaptation than is required to saturate the receptors with green light (e.g. see Fig. 3).

The transition between the fast and slow declining responses is not clearly understood. It is examined in chapter 4. This chapter is concerned with the slow-declining response in the fully blue-adapted eye.

There is a striking similarity between the data associated with blue-adaptation which was obtained using extracellular electrodes (e.g. Fig. 1 in Cosens and Wright, 1975; Fig. 1 in Minke et al., 1975) and that obtained using intracellular electrodes presumed to be in R1-R6 (Fig. 1 in Minke et al., 1975); and also between the interpretations of these phenomena (ERG evidence based on spectral sensitivity measurements: Cosens and Wright, 1975; ERG evidence based on data from morphological mutants reported in Harris et al., 1976: Stark, 1975; ERG evidence and intracellular recordings: Minke et al., 1975). These similarities strongly suggest that both recording techniques yield measures of the same transmembrane events occurring across the membranes of R1-R6.

A considerable amount of evidence is accumulating which indicates that the light induced depolarisation of invertebrate photoreceptors is caused by an influx of sodium ions which results from a light- induced increase in the permeability to this ion across the transducing membrane (e.g. see Wulff et al., 1975 for references to work on Limulus). In addition, Brown and Cornwall (1975) have recently shown that the PDA observed in the Balanus photoreceptors is accompanied by prolongation of such light-induced permeability to sodium. This evidence naturally leads to the hypothesis that a PDA results from the failure of membrane conductance channels to close

following the metarhodopsin generating stimulus. On this interpretation the fast and slow decline of the transmembrane afterpotential (visualised in the ERG or in intracellular recordings) must reflect differences in the rate of closure of these channels.

A convenient explanation of these differences in the rate of dark-closure of the conductance channels (and hence an explanation of both transduction and the PDA) may be constructed from the "excitor-inhibitor" hypothesis proposed to explain the PDA observed in Balanus and Limulus (Hochstein et al., 1973; Minke et al., 1973b) and there are, in addition theoretical reasons for believing that transmitters of some kind are involved in the excitation of invertebrate, rhabdomeric, photoreceptor membranes (for a review see Cone, 1973). However, as I have noted before, the present data on the PCNA in Drosophila provides no evidence which corroborates the "excitor-inhibitor" model in R1-R6 of Drosophila. The principle point of departure between the behaviour of R1-R6 in Drosophila and of the Balanus and Limulus preparations is the absence of an obvious disparity between the dark-decline of the PCNA of a fully blue-adapted eye and the inferred changes in the visual pigment composition. The data presented here (Fig. 10b and text) confirm that the blue-adapted state persists for several hours in Drosophila (sensitivity data from the ERG: Cosens and Briscoe, 1972; duration of PCNA and PDA: Minke et al., 1975). The duration of the PCNA/PDA in Drosophila R1-R6 contrasts markedly with the brevity of the PDAs reported in other species. The fact that large afterpotentials are measurable several hours after blue-adaptation and that a further blue stimulus will again blue-adapt the eye of Drosphila (i.e. reduce the response amplitude and increase the size of the PCNA: Fig. 10b and text) argues that the decline of the afterpotential in this

species accompanies the decay of metarhodopsin M580, or, alternatively that it proceeds more slowly than the changes in the visual pigment composition. The latter of these possibilities is unlikely since an orange stimulus will orange-adapt the eye (i.e. eliminate the PCNA and restore the size of the response amplitude: Fig. 10b and text) even after the eye has been in the blue-adapted state for several hours.

The data presented thus confirm the long time-constant for the dark-decay of metarhodopsin M580 in R1-R6 of Drosophila (6 h: Pak and Lidington, 1974) and all the evidence indicates that in this species the decline of the PCNA and PDA is dependent upon the decay of M580. It follows that there is a component of the membrane conductance (to sodium?) of R1-R6 which is correlated with the number of molecules of metarhodopsin M580 which are present in the receptor membranes of these cells.

The hypothesis advanced here, that there is a component of the membrane conductance of R1-R6 of Drosophila which is correlated with the number of M580 molecules in the receptor membranes, is supported by the following additional evidence.

1. The phenomena of blue and orange adaptation are overt in white-eyed Drosophila (Cosens and Briscoe, 1972; Cosens and Wright, 1975; Stark, 1975; Minke et al., 1975) but not readily observed in the red-eyed wildtype (Cosens and Briscoe, 1972). In vivo and in vitro studies of the visual pigments of white-eyed Drosophila reveal that blue light causes the production of metarhodopsin M580 while orange light eliminates M580 and regenerates rhodopsin R480 (Ostroy et al., 1974; Pak and Lidington, 1974; Harris et al., 1976): similar conversions have not been replicated in red-eyed wildtype flies. The

reason for this is presumably that extensive R to M conversion is prevented in the wildtype by the red screening pigments (Cosens and Briscoe, 1972). In fact the PCNA can be replicated in wildtype Drosophila using "whole field" illumination which presumably allows R to M conversion to occur in most of the ommatidia of the eye (chapter 5).

2. The effects of blue and orange adaptation are cumulative (Figs. 1, 2, 3, 4, 5, 8 and 9) as are the accumulation (using blue light) and the elimination (using orange light) of M580 in white-eyed Drosophila (Harris et al., 1976).

3. White-eyed Drosophila with a reduced amount of visual pigment show reduced PCNAs (Stark and Zitzmann, 1976). A similar finding in respect of the PDA is visible in the records from Calliphora (Razmjoo and Hamdorf, 1976). Stark and Zitzmann (1976) place a different interpretation upon their result, they argue that the "blockage of the inactivation of R1-R6 by vitamin A deprivation" is evidence for the presence of "excitors" and "inhibitors". New evidence presented in chapter 4 casts doubt on this interpretation, and their result represents strong corroboration of the present model.

The present "M580-correlated coductance" model of the afterpotential need not conflict with the "excitor-inhibitor" model but does not call upon its many unquantified parameters. It has the clear advantage that is is experimentally testable because its variables are biophysically defined. The present model does, however, imply that "excitors", if present, have a lifetime exceeding that of metarhodopsin state of the visual pigment. That is, they have a time

constant of decay of several hours, or alternatively, that "excitors" latch open certain conductance channels which remain open until they are "unlatched" by "inhibitors". Applying the rule of parsimony, and bearing in mind the recent evidence of an "opsin-signal" associated with bovine visual pigment (Montal et al., 1977) and the "metarhodopsin-signal" in R1-R6 of Drosophila (see chapter 1; Wong et al., 1976), the value of the "excitor-inhibitor" model in describing the biophysics of R1-R6 in Drosophila is seriously called into question.

Whatever the intervening mechanism, the persistent conductance increase associated with the presence of the metarhodopsin M580, and the resultant reduction in response amplitude (the afterpotential effect: Figs. 2, 7 and text) may account for the "supression" of rhodopsin by metarhodopsin reported in Calliphora (Razmjoo and Hamdorf, 1976). This hypothesis is tested in chapter 3 using Razmjoo and Hamdorf's data. The analysis shows that their data violates the assumption upon which their model is built. Furthermore the qualitative and quantitative nature of the violation is consistent with the afterpotential hypothesis advanced here.

4.2: Interpreting the Afterpotential Effect

The interpretation of the observed correlations between the response amplitude and the size of the ERG afterpotential (Figs. 2 and 7) depends on what exactly the ERG is measuring. If, as has already been suggested, the changes visible in the ERG of white-eyed Drosophila during blue and orange adaptation reflect events occurring across the membranes of R1-R6, then calculations using the type of analogue of the transducing membrane described by Shaw (1968) should account for the afterpotential effect. Shaw's type of analogue has gained

considerable respect in the interpretation of intracellular data (e.g. see Laughlin, 1975) but does not seem to have been applied to ERG data. The modified model used (see next section) assumes that the light induced conductance to sodium across the transducing membrane is proportional to a power function of light intensity. As with the original model, a sigmoid relationship between transmembrane potential and the logarithm of the intensity of the stimulus is predicted (e.g. Shaw, 1968). On the assumption that the afterpotential can be accounted for by an "afterpotential equivalent stimulus", calculations with the model reveal an expected curvilinear negative correlation between the response to a fixed intensity stimulus and the afterpotential associated with an independent source of conductance, and that this relationship becomes increasingly linear as the intensity of the stimulus is increased. This is the relationship reported in the results section (e.g. see Fig. 5 and accompanying text). It also follows, directly from the model that an exponential decline of conductance results in a sigmoid decline in the afterpotential: in the results section a sigmoid declining afterpotential was observed when the metarhodopsin M580 was exponentially eliminated (Figs. 4, 5, 6). It appears that in white-eyed Drosophila (where the retinula cells of all of the ommatidia tend to respond in concert) the membrane analogue will describe data from the ERG as it does data from intracellular recordings (Shaw, 1968; Laughlin, 1975). It is implied that, at least in the particular experimental situation of blue/orange adaptation, the ERG of white-eyed Drosophila is revealing events which occur across the transducing membrane of photoreceptor cells.

4.3: A Model of the Afterpotential

The input-output equation for sensory receptors is given by Lipetz (1969) as

$$V/Vmax = x/(x + 1) \qquad \text{(eqn. 4.3-1)}$$

where $x = (I/sigma)^n$ and sigma is the value of I when $V/Vmax = 0.5$

This relatonship between input and output can be obtained from a model sensory receptor such as that shown in Figure 12. The model works on the assumption that light intensity I modulates the value of the sodium conductance gNa. The equation for the voltage output of this model is

$$V/Vmax = (Vm - Ek)/(ENa - Ek) = x/(x + 1) \qquad \text{(eqn. 4.3-2)}$$

where $x = gNa/gk$ (see Shaw, 1968; Naka and Rushton, 1966)

In order to make this model match with the mathematical description given by Lipetz, it is necessary to put

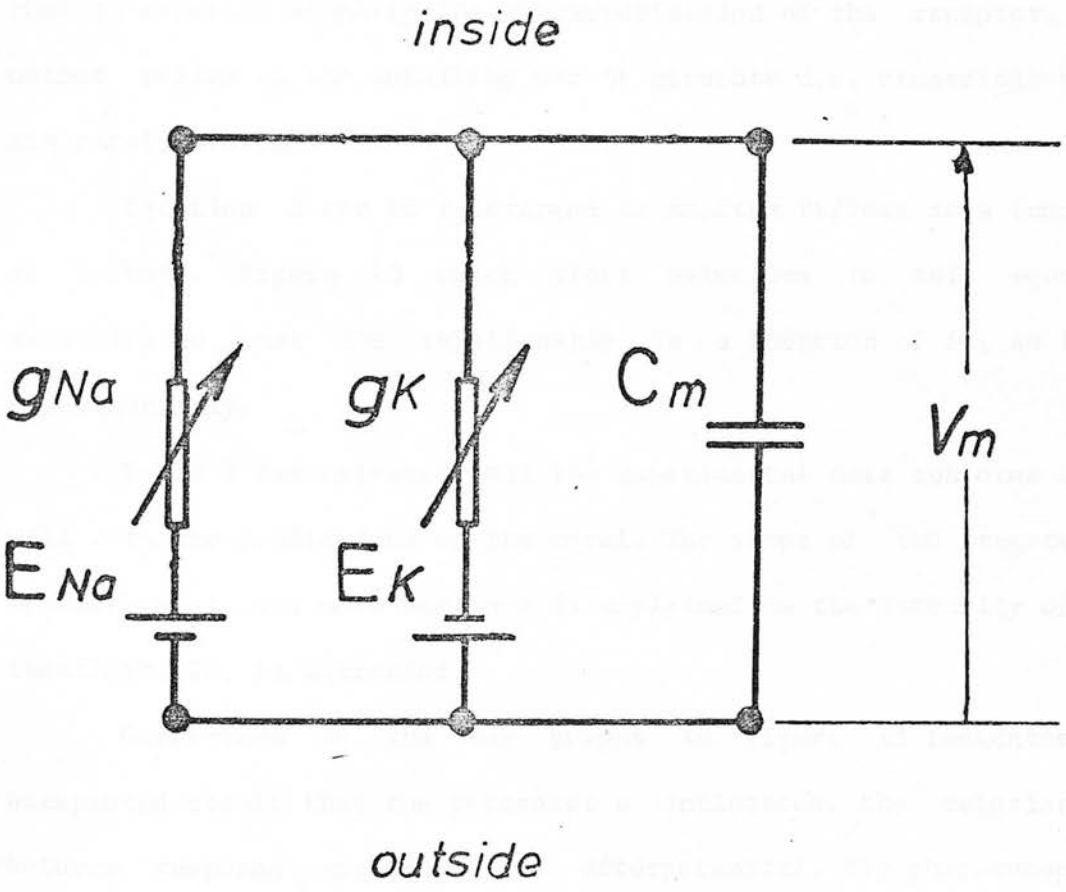
$$gNa = (I/sigma)^n/gK$$

It is most convenient to use equation 1 in the familiar form

$$V/Vmax = I^n/(I^n + sigma^n) \qquad \text{(eqn. 4.3-3)}$$

In the previous section it was assumed that an afterpotential was due to a component of sodium conductance as is the stimulus

Figure 12: A model of the membrane of a sensory receptor used by Naka and Rushton (1966), Shaw (1968) and others which is adopted here as a model of R1-R6 in Drosophila. The model is derived from the parallel conductance model of the axon (Hodgkin, 1964) and is fully described in the textbooks (e.g. Kuffler and Nicholls, 1976, Junge, 1976).



coincident response. If this is the case, then an afterpotential can be considered to be due to an "afterpotential equivalent stimulus" (Ia) and its voltage can be represented simply as Va. If the intensity of the real testflash stimulus is designated It and the response amplitude by Vt, then the TOTAL voltage response is V = (Vt + Va), and the TOTAL "effective" stimulus is I = (It + Ia).

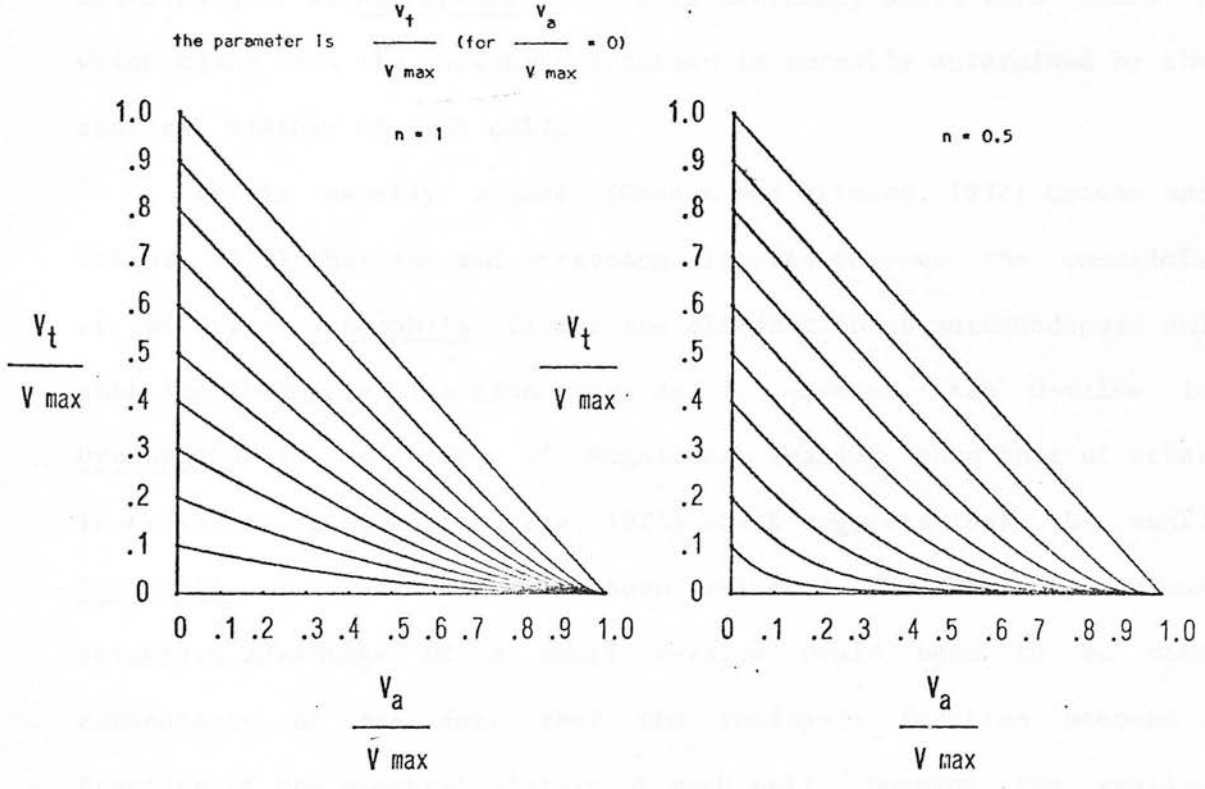
This summation of two component voltages to give a total voltage, and two component stimuli to give a total stimulus, is similar to the treatment used by Laughlin (1975) for "adapting" and "test" stimuli. In the case of afterpotentials, an imaginary adapting light Ia can be assumed. The difference between the present treatment and that of Laughlin - apart from the inclusion of the parameter n which proves to have important consequences - is that here account is taken of the fact that the two voltages Va and Vt are separately experimentally measurable. Laughlin (1975) always lumped these together in order to simplify the characterisation of the receptor. His method relies on the unfailing use of accurate d.c. recordings which are rarely available.

Equation 3 can be rearranged to express Vt/Vmax as a function of Va/Vmax. Figure 13 which plots solutions to this equation demonstrates that the relationship is a function of It, as found experimentally.

Table 3 demonstrates that the experimental data conforms quite well with the predictions of the model. The slope of the regression approaches -1, and more variance is explained as the intensity of the testflash, It, is increased.

Comparison of the two graphs in Figure 13 indicates the unexpected result that the parameter n influences the relationship between response amplitude and afterpotential. Fly photoreceptors

Figure 13: Response amplitude (Vt) is plotted as a function of the afterpotential (Va) according to the model of equation 4.3-3 assuming that the afterpotential is due to an equivalent stimulus Ia calculated from this equation. Response amplitude and afterpotential are expressed as fractions of Vmax. It was assumed in the calculation that n and sigma do not vary. The two graphs show results obtained for two values of n: n = 1 and n = 0.5. The function on each graph is plotted for ten values of the response amplitude when the afterpotential is zero. The model predicts a negative correlation between response amplitude and afterpotential and that the slope of the relationship becomes more linear and approaches -1 as Vt increases.



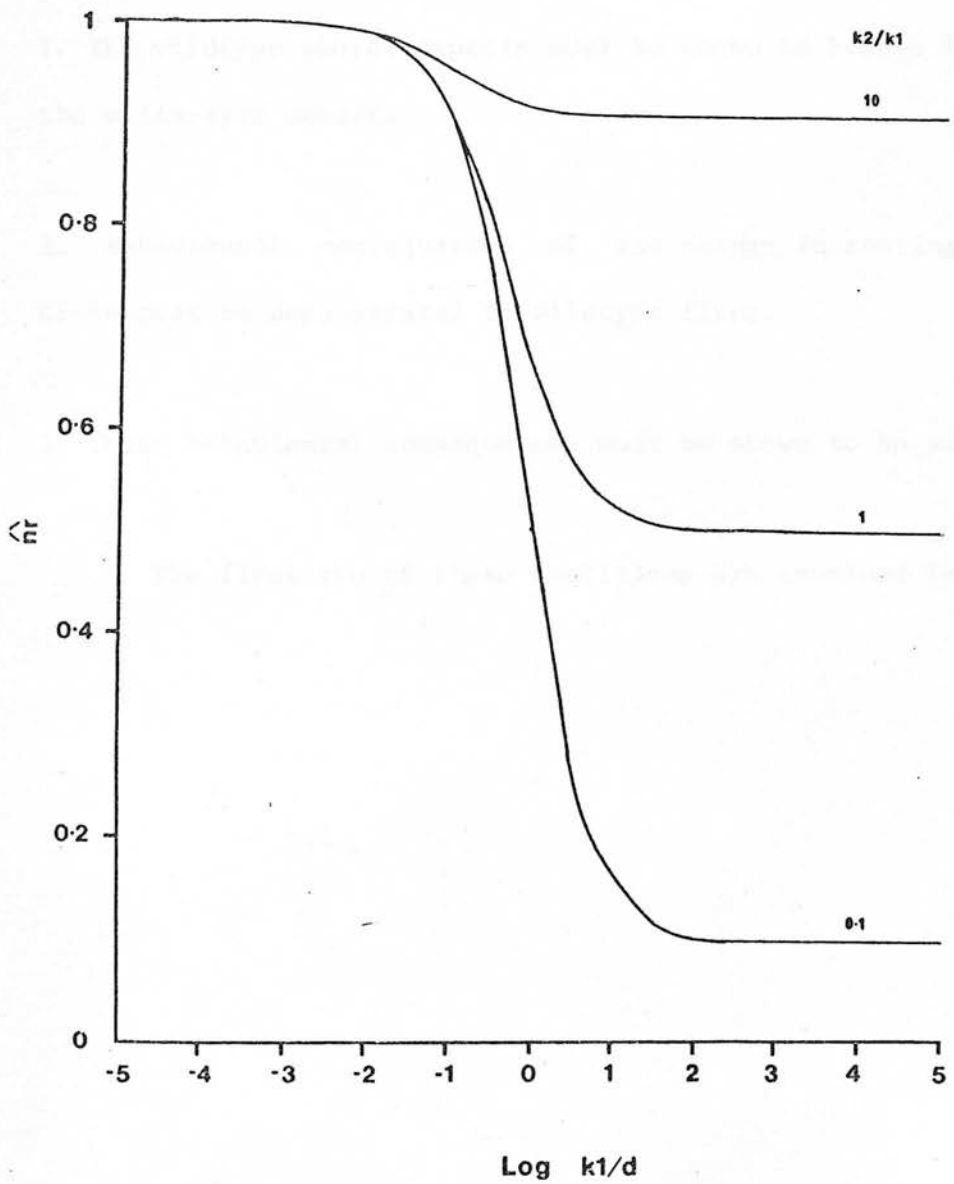
have an n-value of about 0.5 (e.g. Drosophila: Minke et al., 1975; Calliphora: chapter 3).

4.4: The Design of the Visual Pigment System in R1-R6 of Drosophila

Figure 14 shows the equilibrium value for the fraction of the visual pigment in the rhodopsin state as a function of the ratio k1/d, calculated from equation 3.1-1 given at the beginning of the results section. The parameter is the ratio k2/k1, which depends upon the wavelength of light. The figure shows that when k1 and k2 are less than 0.001d the fraction of visual pigment in the rhodopsin state never goes below 0.98. That is, a high value of rhodopsin concentration is maintained under all lighting conditions. On the other hand, when k1 is greater than 100d, the rhodopsin fraction is determined almost entirely by the ratio k2/k1 and hence by the wavelength of illumination. In Drosophila R1-R6 d is extremely small (1/6 hours^{-1}) which means that the rhodopsin fraction is normally determined by the spectral history of each cell.

It is usually argued (Cosens and Briscoe, 1972; Cosens and Wright, 1975) that the red screening pigments between the ommatidia of wildtype Drosophila favour the elimination of metarhodopsin and push the rhodopsin fraction towards 1. However, the d-value in Drosophila is an order of magnitude smaller than that of other invertebrate species (Stavenga, 1975) which suggests that the small Drosophila d-value may have been selected for. The only obvious selective advantage of a small d-value would seem to be some consequence of the fact that the rhodopsin fraction becomes a function of the spectral history of each cell. Because the resting potential of R1-R6 depends on the quantity of metarhodopsin present

Figure 14: The figure shows the fraction of visual pigment in the rhodopsin state when equilibrium is reached with the incident light. The rhodopsin fraction ($\hat{nr}$) was calculated using equation 3.1-1 and the figure shows the interaction of the wavelength and the intensity of light in their effect on $\hat{nr}$. The parameter k2/k1 is wavelength dependent but independent of intensity. The X-axis represents the logarithm of the intensity of light. The scale is drawn in units of k1/d. That is, zero on the scale corresponds to that intensity of light at which the forward reaction R to M is exactly balanced by the dark reaction M to R.



in the membranes of R1-R6, and the change in resting potential alters the behaviour of the cells, the rhodopsin fraction has a direct input to the peripheral nervous system. Hence each cell contains information about its previous history.

The design of the visual pigment system in R1-R6 of Drosophila may thus represent a compromise between (1) the maintainance of a high rhodopsin fraction leading to high sensitivity and (2) the maintainance of a molecular and neural correlate of the previous history of the cell, with the attendant possibility of adaptive behavioural modification.

If this hypothesis is to be corroborated three conditions must be fulfilled

1. The wildtype photoreceptors must be shown to behave like those in the white-eyed mutant.

2. Behavioural consequences of the change in resting potential of R1-R6 must be demonstrated in wildtype flies.

3. These behavioural consequences must be shown to be adaptive.

The first two of these conditions are examined in chapter 5.

CHAPTER 3

THE EFFECT OF AFTERPOTENTIALS ON V-LOG I CURVES

1: INTRODUCTION

In chapter 2 the effect of afterpotentials upon the behaviour of sensory receptors was examined theoretically and experimentally using the ERG of white-eyed Drosophila. It was found experimentally that there is a negative correlation between the size of the afterpotential and the response amplitude. This behaviour was found to be a property of a generalised model of the receptor cell membrane. No previous report has considered the effect of membrane potential upon the response amplitude but abortive attempts have been made to correlate membrane potential with "sensitivity" (e.g. Fein and DeVoe, 1974). "Sensitivity" in this case was defined as the inverse of the amount of light required to elicit a criterion response increment. This "sensitivity" parameter is obtained by plotting the response amplitude against the logarithm of the stimulus intensity, the afterpotential being ignored (e.g. Hamdorf and Schwemer, 1975; Razmjoo and Hamdorf, 1976).

This chapter simulates the effect of afterpotentials upon the classic V-log I curve. Three methods of plotting the results are used. These correspond to methods used in the literature for plotting experimental data: (1) with the afterpotential included; (2) with the afterpotential ignored or overlooked (e.g. Fein and DeVoe, 1974; Razmjoo and Hamdorf, 1976); (3) with the afterpotential ignored and all curves "normalised" to a common range on the V-axis. Yet another alternative has been offered by Laughlin (1975).

The simulated results are compared with a statistical analysis of data from R1-R6 of Calliphora which has been used by its collectors to propose the existence of a molecular interaction between rhodopsin and metarhodopsin when the concentration of metar-

hodopsin is high (Razmjoo and Hamdorf, 1976). The analysis shows that the assumptions of Razmjoo and Hamdorf are violated, their model thus falls. Their data is shown to be consistent with an overlooked metarhodopsin-correlated afterpotential.

2: METHODS

2.1: Statistical Analysis

Selected data from Figure 3a of Razmjoo and Hamdorf (1976) was fitted to the equation

$V/Vmax = I^n/(I^n + sigma^n)$ (eqn. 4.3-3: chapter 2)

using the method of Newton-Gauss iterations (program BMDP3R: BMD Manual, 1975). This method fits parameters to a user specified differentiable function such as eqation 4.3-3. It is a least squares non-linear regression method.

2.2:Simulation

Solutions to the equation above were plotted assuming V = (Vt + Va) and I = (It + Ia) as in chapter 2. The equation was solved for ten values of Va and two values of n. The curves were ploted in the three formats mentioned in the introduction using a Hewlett Packard 9820 calculator and a graph plotter peripheral.

3: RESULTS

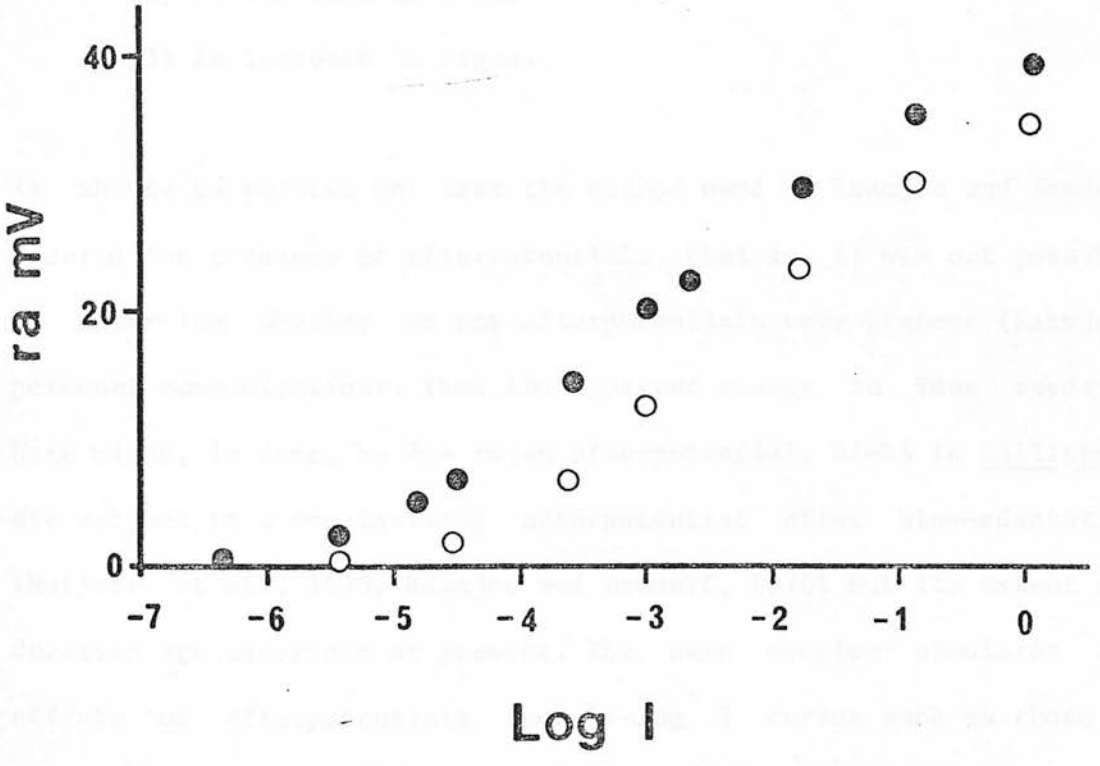
3.1: Statistical Analysis

Figure 1 shows the data taken from Figure 3a of Razmjoo and Hamdorf (1976). The two curves were obtained from liver-fed (high visual pigment) chalky-eyed Calliphora. The solid circles show data from the fly after red-adaptation when the rhodopsin fraction was maximal: nr = 1. The open circles show data from the same fly after blue-adaptation when nr was approximately 0.2 (Razmjoo and Hamdorf, 1976). According to Razmjoo and Hamdorf the curves defined by the two sets of circles are parallel, that of the blue-adapted state shifted along the log I axis by 7.2 units of I. They use this shift in the intensity of light required to elicit an equivalent voltage response as a definition of a change in "sensitivity", which, using equation 1, corresponds to a change in the parameter sigma if the curves are parallel.

The "sensitivity" hypothesis of Hamdorf and Schwemer (1975) states that "sensitivity" is directly proportional to the quantity of rhodopsin present (or in this case nr). The "sensitivity" shift predicted is thus approximately 4. Razmjoo and Hamdorf (1976) found a "sensitivity" shift of 4.6 in heart fed (low visual pigment) Calliphora which they argue is in agreement with the predicted value of 4. The clear discrepancy in the liver-fed (high visual pigment) fly, the argue, is due to an additional "sensitivity" shift found only when large quantities of metarhodopsin are present resulting in ".....a loss of effectivity of half of the excited rhodopsin molecules".

Their explanation is plainly ad hoc. The need for it rests on the validity of their stated assumpton that the two V-log I curves are parallel. This assumption is tested below.

Figure 1: Data replotted from Razmjoo and Hamdorf (1976; Figure 3a). The curves show the response amplitude (ra) against the logarithm of the stimulus intensity (log I) from vitamin A enriched Calliphora (intracellular recordings from R1-R6). Solid circles: data from red-adapted fly with rhodopsin concentration maximal (nr = 1). Open circles: data from blue-adapted fly with nr = 0.2. Razmjoo and Hamdorf state that the curves defined by the two sets of points are parallel but shifted in sensitivity by a factor of 7.2. The data is statistically analysed to test these assertions in this chapter.



If the points on Figure 1 fall on parallel curves equations of the form of eqn. 4.3-3 fitted to the points will differ only in the parameter sigma (see chapter 1 Fig. 3). The results of the statistical analysis are given in Table 1. For both sets of points the variance explained by the regression is greater than 99% showing that the equaion used adaquately describes the data. However, the Table shows results contrary to the interpretation of Razmjoo and Hamdorf: all three parameters, Vmax, n and sigma are significantly diffent between the treatments.

The analysis shows that conversion of 80% of the visual pigment in R1-R6 of Calliphora results in

(1) a reduction of Vmax,

(2) an increase in n and

(3) an increase in sigma.

It should be pointed out that the method used by Razmjoo and Hamdorf ignored the presence of afterpotentials, that is, it was not possible to determine whether or not afterpotentials were present (Razmjoo, personal communication). Thus the apparent change in Vmax reported here might, in fact, be due to an afterpotential. R1-R6 in Calliphora are subject to a depolarising afterpotential after blue-adaptation (Muijser et al., 1975; Razmjoo and Hamdorf, 1976) but its extent and duration are uncertain at present. The next section simulates the effects of afterpotentials upon V-log I curves such as those in Figure 1 and shows that the parameter changes observed in the Calliphora data (Table 1) may be entirely due to an afterpotential.

Table 1. Parameter values of the equation $V/Vmax = I^n/(I^n + \sigma^n)$ fitted to the data from Calliphora

R_{1-6} red-adapted; nr = 1

df = 6	t-value	
Vmax = 44.7 ± 2.01 (S.E.)	21.6	+++
n = 0.46 ± 0.035	13.2	+++
σ = 0.0014 ± 0.00034	3.62	+
Variance explained by regression = 99.46%		+++

R_{1-6} blue-adapted; nr = 0.2

df = 4

Vmax = 34.85 ± 0.62	56.2	+++
n = 0.57 ± 0.026	21.96	+++
σ = 0.0032 ± 0.0004	8	+++
Variance explained by regression = 99.93%		+++

Parameter difference (blue-adapted - red-adapted)

df = 4	t'-value	
Vmax = -9.85	5	++
n = 0.11	4.7	++
σ = 0.0018	20.1	+++
σ - ratio = 2.27		

+ = $p < .025$ ++ = $p < .01$ +++ = $p < .001$

To distinguish between parameter changes independent of changes in the size of the afterpotential and those caused entirely by the afterpotential, the latter will be called apparent or "pseudo" parameter changes.

3.2: Simulation

Figure 2 shows the results of the simulation when the parameter n takes the value 1. Figure 3 shows the results when n = 0.5 which is approximately the value appropriate to the Calliphora data (Table 1). The Figures show the results plotted (A) with the afterpotential included, (B) with the afterpotential ignored (as in Fig. 1) and (C) with the afterpotential ignored and the curves "normalised" to a common range on the V-axis.

When n = 1 afterpotentials induce the following "pseudo" parameter changes if afterpotentials are ignored.

(1) A reducton in Vmax.

(2) An increase in the point of inflection of the curve (i.e. sigma)

(3) No change in n.

When n = 0.5 afterpotentials induce the following "pseudo" parameter changes.

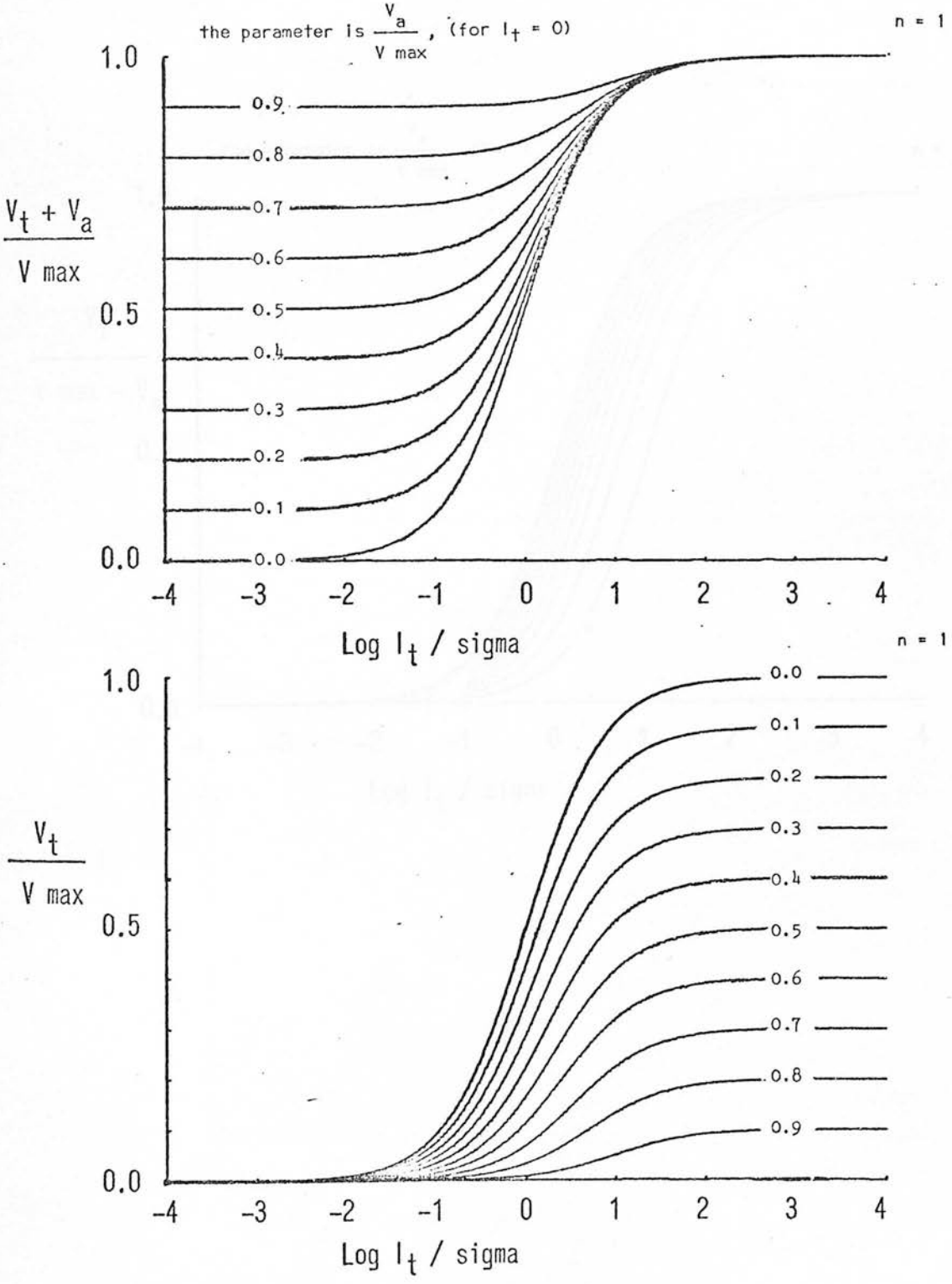
(1) A reduction in Vmax

(2) An increase in sigma

(3) An increase in n

Thus when n is approximately 0.5, as it is in R1-R6 of Calliphora (Table 1), afterpotentials induce, qualitatively, exactly the changes

Figure 2: The results of simulations of the effect of afterpotentials on V-log I curves. The results of the simulation are plotted in the three formats described in the introduction. This figure shows the results of the simulation when n = 1. See section 3.2 for discussion.



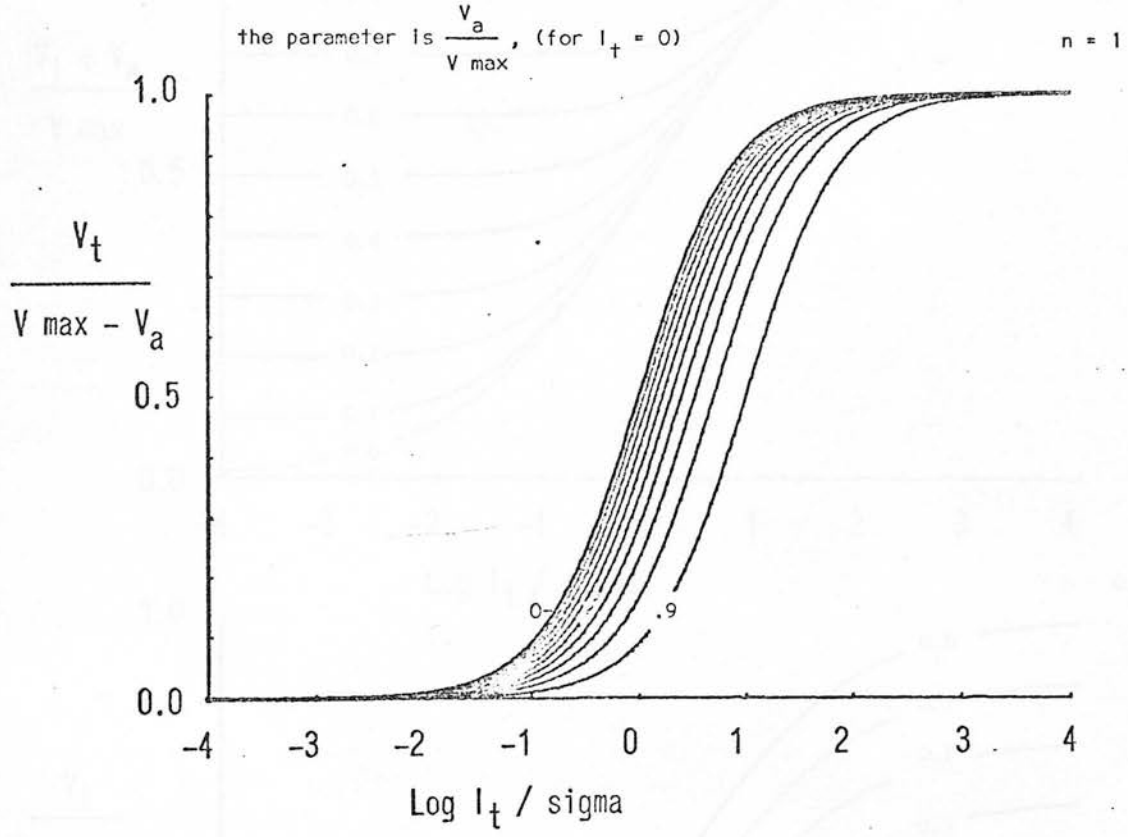
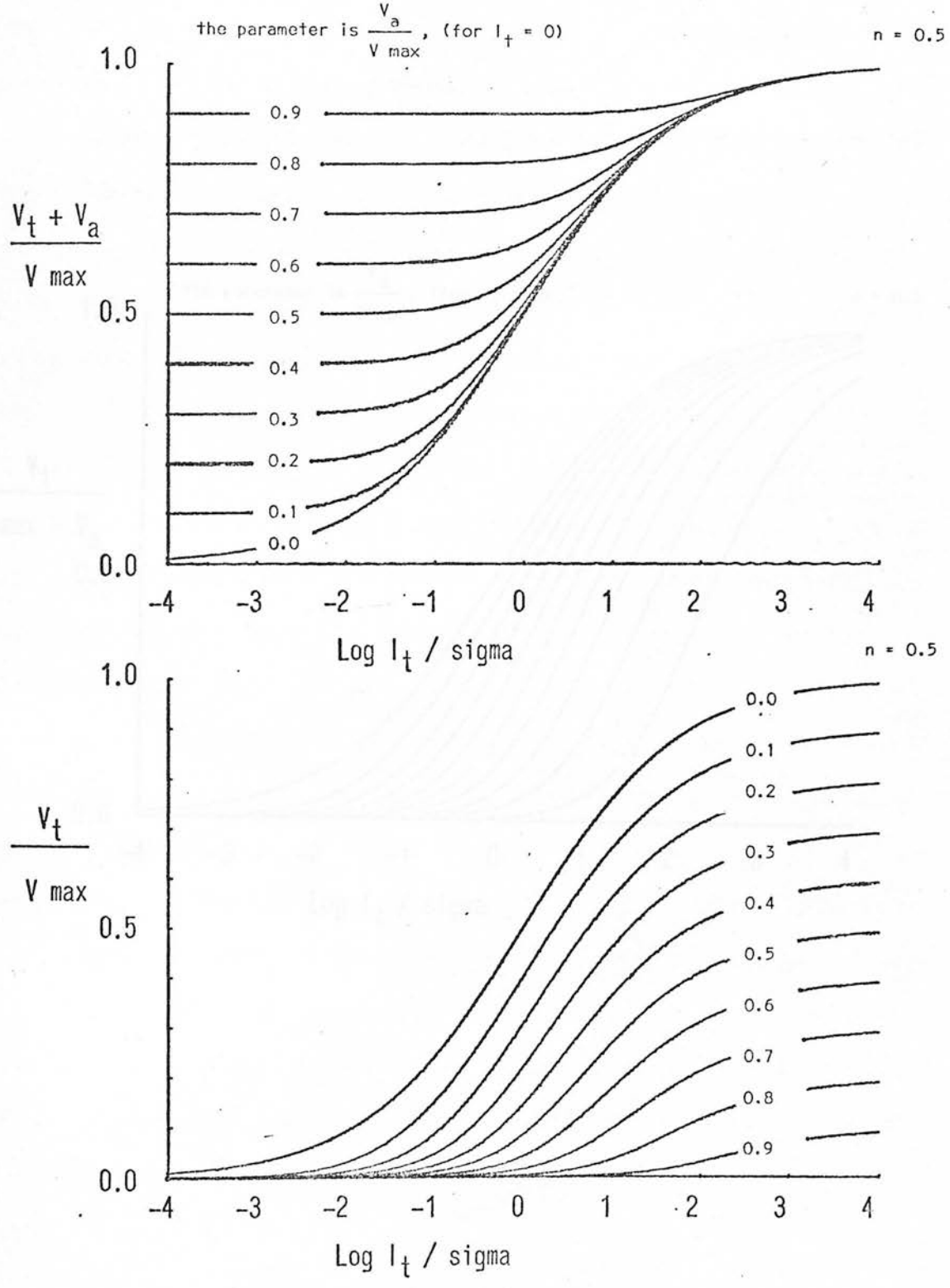
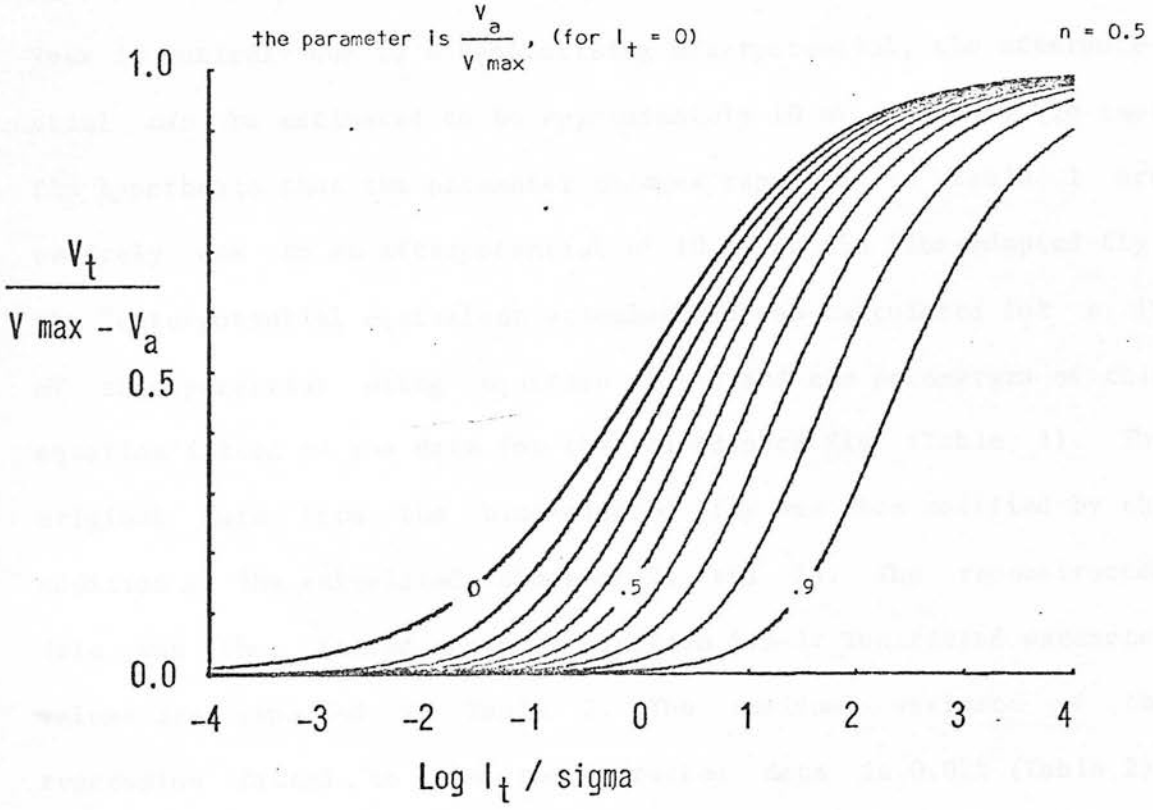

the parameter is $\frac{V_a}{V\ max}$, (for $I_t = 0$)
n = 1
1.0
$\frac{V_t}{V\ max - V_a}$
0.5
0.0
0-
.9
-4
-3
-2
-1
0
1
2
3
4
Log I_t / sigma

Figure 3: As figure 2 except that n = 0.5.




the parameter is $\frac{V_a}{V\ max}$, (for $I_t = 0$)
n = 0.5
1.0
$\frac{V_t}{V\ max - V_a}$
0.5
0.0
0
.5
.9
-4
-3
-2
-1
0
1
2
3
4
Log I_t / sigma

observed after blue-adaptation (Fig. 1; Table 1). The next section examines these changes quantitatively.

3.3: Statistical Analysis Assuming an Afterpotential Accounts for the Change in Vmax Observed After Blue-adaptation

The absence of direct measurements of the afterpotential in the blue-adapted Calliphora data makes it impossible to determine directly which of the parameter changes reported in Table 1 are real and which are "pseudo" parameter changes due to any afterpotential that may have been present. On the assumption that the change observed in Vmax is entirely due to a depolarising afterpotential, the afterpotential can be estimated to be approximately 10 mV (Table 1). To test the hypothesis that the parameter changes reported in Table 1 are entirely due to an afterpotential of 10 mV in the blue-adapted fly, the "afterpotential equivalent stimulus" Ia was calculated for a 10 mV afterpotential using equation 4.3-3 and the parameters of this equation fitted to the data for the red-adapted fly (Table 1). The original data from the blue-adapted fly was then modified by the addition of the calculated values of Va and Ia. The reconstructed data was then fitted again to equation 4.3-3. The fitted parameter values are reported in Table 2. The residual variance of the regression fitted to the reconstructed data is 0.02% (Table 2), marginally less than the value of 0.07% for the original curve (Table 1). The parameter differences between red and blue adapted states of the eye are no longer significantly different using the reconstructed data on blue-adaptation (Table 2), a result which is consistent with the hypothesis advanced here.

Table 2. Parameter values in the equation $V/Vmax = I^n/(I^n + \sigma^n)$ fitted to the data from blue-adapted Calliphora adjusted assuming an afterpotential of 10 mV from which Ia is calculated using the equation fitted to the curve from the red-adapted state (Table 1).

df = 4	t-value	
Vmax = 45.7 ± 0.529 (S.E.)	86.4	+++
n = 0.46 ± 0.014	32.86	+++
σ = 0.00127 ± 0.00036	3.53	+

Variance explained by regression = 99.98%

Parameter difference (blue-adapted - red-adapted)

	t'-value	
Vmax = 1	0.47	NS
n = -0.002	0.053	NS
σ = -0.00013	0.255	NS

NS = not significant

4: DISCUSSION

The simulations demonstrate theoretically the effect of afterpotentials upon V-log I curves. The data of Razmjoo and Hamdorf (1976) from blue-adapted Calliphora is shown to correspond qualitatively and quantitatively to the presence of a depolarising afterpotential which may account for all of the observed differences between red-adapted and blue-adapted Calliphora when the visual pigment content of R1-R6 is high.

It would be rash to assume that the quantitative agreement between the Calliphora data and the hypothesis arising from the study of d.c. potentials in the ERG of Drosophila (Chapter 2) indicates that the hypothesis explains the data completely. There are many other adaptation processes proceeding in parallel at any time. The analysis does, however, illustrate some points of importance.

1. Failure to use a mathematical description of V-log I curves has led to interpretations which are rendered redundant by mathematical analysis of the original data.

2. Statistical fit of the curves supplied by Razmjoo and Hamdorf (1976) reveals a sigma value change which is smaller, not larger, than the change predicted by Hamdorf and Schwemer's "sensitivity" hypothesis (Table 1).

3. No evidence in support of either Hamdorf and Schwemer's or Razmjoo and Hamdorf's "sensitivity" models is found in the data from high visual pigment Calliphora.

4. In the absence of d.c. recordings no definitive statement about mechanisms of adaptation can be based on the shape of V-log I curves because afterpotentials have effects on all parameters of the standard equation of receptor response (see also Glantz, 1972).

5. When afterpotentials may be present there is no unique variable such as "sensitivity" which can completely characterise adaptation and which is independent of afterpotential magnitude.

6. The equations developed in chapter 2 appear to be the only ones available which are competent to incorporate afterpotentials even if they are not measured (e.g. Table 2), ideally, however, afterpotentials should always be recorded as the method used in Table 2 is somewhat empirical and inconclusive.

As a final note here, I would simply observe that the dependence of the "pseudo" parameter sigma upon the size of the afterpotential is highly non-linear, which presumably explains the failure of Fein and DeVoe (1974) to find a perfect linear correlation. If afterpotentials are measured, and they are incorporated into the equations of chapter 2, all of the other parameters of the equations are independent.

The only consistent way to describe the behaviour of a receptor is in terms of the parameters of an objectively fitted equation. The equations given in chapter 2 are considerably more parsimonious than those of Glantz, which ignore afterpotentials in the final reckoning and result in confusing "pseudo" parameter changes (Glantz, 1972; see Laughlin, 1975 for critiscism of Glantz' treatment of "light-adaptation").

CHAPTER 4

BRIEF DEPOLARISING AFTERPOTENTIALS IN THE ERG OF *DROSOPHILA*

1: INTRODUCTION

Chapter 2 was concerned with the saturating PCNA observed in the ERG of Drosophila after exposure of the eye to large doses of blue light. This chapter describes the behaviour of the ERG afterpotential after smaller quantities of blue light have been delivered to the eye.

2: METHODS

The materials and methods used were identical to those reported in chapter 2. Details of the illumination procedures used in individual experiments are given in the appropriate place in the text or in the figure legends.

3: RESULTS

3.1: Kinetics of the Afterpotential

Two features of the ERG afterpotential alter as increasing quantities of blue light are delivered to the eye: (a) the rate of decay of the afterpotential and (b) the lower asymptote of this decay (Fig. 1; Table 1). Figure 1 shows the decline of afterpotential following different doses of blue light delivered in equal intensity flashes. It can be seen that after each dose the afterpotential decays approximately exponentially, but that as the number of flashes delivered increases the asymptote of this decay becomes larger.

Table 1 reports a quantitative analysis of records obtained from the preparation used to construct Fig. 1. The equation for an

Fig. 1: The figure shows the decay of the afterpotential following different doses of blue light. Different quantities of light were delivered to the eye of the preparation in 1 s pulses from the adapting-flash (intensity: 0.8 X 10^{15} photons cm^{-2} s^{-1}). Doses from 1 to 30 pulses were delivered. The ERG base line was recorded for two minutes after each dose. The figure shows the afterpotential kinetics after three of these doses: 3, 10, and 15 pulses. The data is plotted relative to the ERG baseline before the dose was delivered and the records were aligned in time at the termination of the last pulse of light. After small doses of blue light (e.g. 3 pulses) the ERG baseline appears to decay back to its original level -- thus there is no PCNA, but the time required to decay becomes larger as more blue light has been delivered. After larger doses of blue light (data for 10 and 15 doses are shown) not only does the time required for decay remain high but the ERG now appears to decay to a level higher than its original level - thus a PCNA is visible at the end of the two minute observation period.

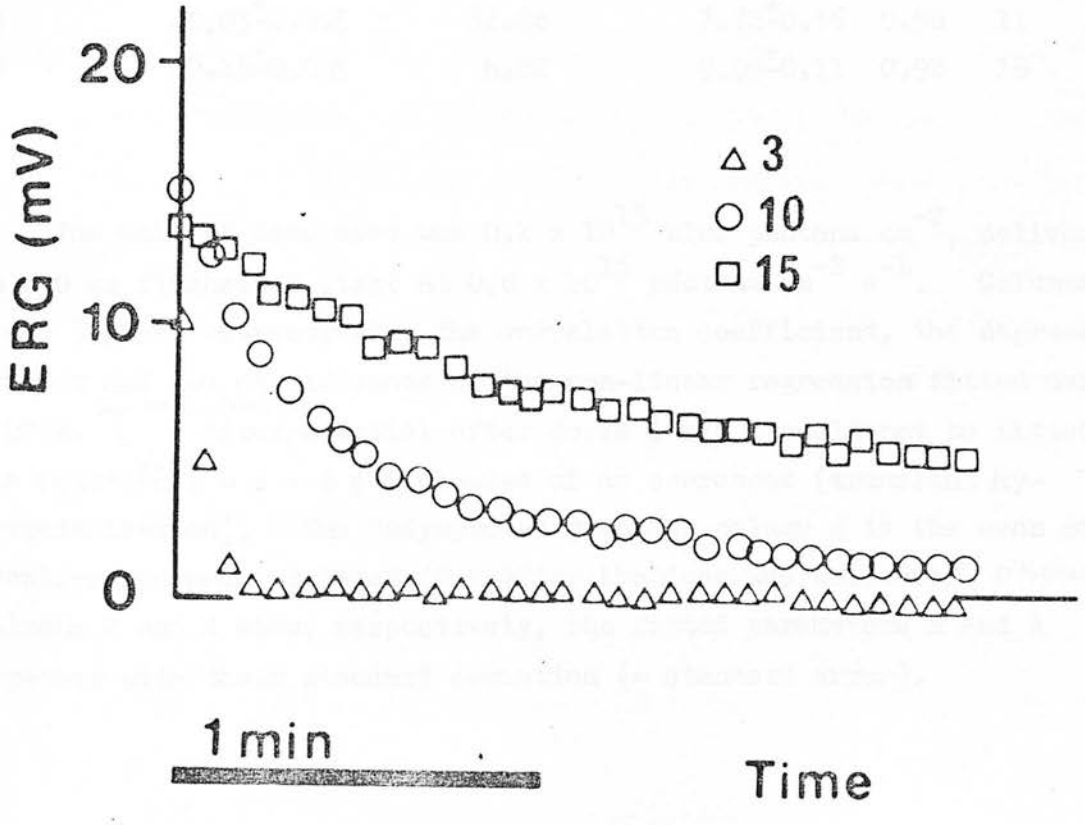


Table 1. The Afterpotential following small doses of blue light.

1	2	3	4	5	6	7
Dose	Rate constant(s^{-1})	Time constant (s)	Asymptote (mV)	r	df (n-3)	p <
1	-	< 4	-0.14 ± 0.19	-	16	-
2	-	< 4	-0.17 ± 0.18	-	12	-
3	-0.21 ± 0.01	4.81	-0.34 ± 0.07	0.98	21	.001
4	-0.18 ± 0.01	5.47	-0.10 ± 0.08	0.99	21	.001
5	-0.08 ± 0.01	11.85	0.39 ± 0.05	0.98	27	.001
6	-0.07 ± 0.004	14.74	1.93 ± 0.09	0.98	30	.001
7	-0.06 ± 0.002	17.97	1.59 ± 0.08	0.99	28	.001
8	-0.04 ± 0.003	22.68	0.42 ± 0.14	0.98	26	.001
9	-0.04 ± 0.002	26.58	1.19 ± 0.11	0.99	27	.001
10	-0.03 ± 0.002	34.53	0.89 ± 0.16	0.99	29	.001
15	-0.02 ± 0.001	62.47	4.10 ± 0.35	0.99	26	.001
20	-0.03 ± 0.005	32.28	7.78 ± 0.36	0.98	11	.001
30	-0.15 ± 0.005	6.82	9.09 ± 0.11	0.92	15	.001

The unit of dose used was 0.2×10^{15} blue photons cm^{-2}, delivered in 250 ms flashes of light at 0.8×10^{15} photons cm^{-2} s^{-1}. Columns 5, 6 and 7 give, respectively, the correlation coefficient, the degrees of freedom and the significance of the non-linear regression fitted using BMDP3R. For these doses the afterpotential after doses 1 and 2 could not be fitted by the equation $y = A + B\,e^{Rx}$ because of an overshoot (transient hyperpolarisation). The "asymptote" given in column 4 is the mean of measurements made at least 30 s after the dose was delivered. Otherwise columns 2 and 4 show, respectively, the fitted parameters R and A together with their standard deviation (= standard error).

exponential decay to an asymptote is $y = A + B\ e^{-Rx}$ (Snedecor and Cochran, 1967). This equation was fitted to the data using a non-linear regression program BMDP3R (BMD manual, 1975). As the correlation coefficients for the equation fitted to the afterpotentials are high (> 0.9), the decays are close to exponential.

The time-constant shown in Table 1 is obtained as the inverse of the rate-constant parameter R fitted by the regression program and is dependent upon the dose of blue light delivered to the eye. Column 5 of Table 1 confirms the subjective impression gained from Fig. 1 that small quantities of blue light, and hence small quantities of generated M580, are followed by afterpotentials which fall to an asymptote which is close to 0 mV. This lag in the development of a PCNA means that the relationship between the magnitude of the PCNA and the quantity of blue light delivered to the eye is sigmoid as implied in chapter 2.

Figure 1 and Table 1 illustrate two important results. 1. Small quantities of blue light do not induce a measurable PCNA. 2. In contrast to those reported in other species, the PCNA/PDA in R1-R6 of Drosophila may be of a magnitude intermediate between that of a saturated PCNA/PDA and the "dark-adapted" level of the ERG baseline.

In order to distinguish the kinetics described here from those of the saturated PCNA, the asymptotic decay of the ERG baseline with a time constant of seconds to minutes (Table 1) will be called the "brief afterpotential".

The brief afterpotential is not accounted for by the model of the M580-correlated conductance which appears to account for the PCNA (chapter 2) because the brief afterpotential declines with a time-constant which is independent of the decay of M580 (the time-constant for the decay of M580 is about six hours: Pak and Lidington, 1974).

The non-linear (sigmoid) relationship between the magnitude of induced PCNA and the quantity of blue light delivered to the eye also implies a sigmoid relationship between the magnitude of the PCNA and the quantity of M580 in the membranes of R1-R6 of Drosophila. The result of Stark and Zitzmann (1976), that it is difficult to observe a PCNA in Drosophila with 0.5% of the normal quantity of visual pigment, is consistent with the present result.

3.2: Suppression of the Brief Afterpotential by Previous M to R Conversion

The brief afterpotential in the Drosophila retina resembles the prolonged depolarising afterpotential in other species (e.g. Limulus and Balanus) in that it decays independently of the decay of its associated metarhodopsin. The PDA in Limulus and Balanus may be prevented (suppressed) by an immediately preceding M to R conversion, the suppression process being called an "anti-tail" (Hochstein et al., 1973; Minke et al., 1973). It is pertinent to ask whether the PCNA or the brief afterpotential in Drosophila may be similarly suppressed by M to R conversion.

In the experiments reported, M to R conversion was achieved by blue-adapting the eye of the preparation with 10 s of the adapting light, and then orange-adapting the eye with 10 s of orange light (the M to R conversion step). This procedure will simply be called the "treatment" in what follows.

No treatment ever produced any visible effect upon the PCNA induced by 10 s of blue light at the maximum intensity available from the adapting light. The present result cannot rule out the possibility that M to R conversion partially suppresses the PDA/PCNA in

Drosophila. It does, however, illustrate that unlike the Balanus and Limulus preparations complete suppression of the PDA in R1-R6 does not occur.

In contrast, suppression of the brief afterpotential by previous M to R conversion can be reliably observed. Ten preparations were examined for suppression of the brief afterpotential effect. All preparations gave positive results: the brief afterpotential declined more rapidly after treatment than after 10 minutes of dark-adaptation. This result is statistically significant ($P<0.01$; binomial test). Controls demonstrated that this suppression is the result of M to R conversion rather than illumination with orange light. Figure 2 is a sample record demonstrating the suppression of the brief afterpotential in the ERG of Drosophila. As in the Balanus and Limulus preparations the suppression effect of an M to R conversion in the Drosophila retina decays with time. Figure 3 is a plot of the half-life of the brief afterpotential against the time after treatment. Linear correlation between these variables was statistically significant ($r = 0.87$, $df = 5$, $P<0.01$), but a much better fit was obtained by correlating the half-life of the brief afterpotential with the logarithm of the time after treatment ($r = 0.98$, $df = 5$, $P<0.01$). The correlation plotted in Fig.3 is the latter of these. My observations indicate that the "anti-tail" is nearly exhausted after 10 minutes.

4: DISCUSSION

The depolarising afterpotential induced in R1-R6 of the Drosophila retina by blue light is shown here to comprise two phases: (a) the brief afterpotential which decays with a time constant of

Fig. 2: Suppression of the brief afterpotential following M to R conversion. The figure shows tracings of the afterpotential which followed a 1 s blue stimulus from the adapting light (intensity: 1.4 X 10^{16} photons cm^{-2} s^{-1}), (a) before blue-adaptation, (b) 5 s after M to R conversion (note that the afterpotential decays more rapidly than in (a)), (c) 1 min, and (d) 5 min after M to R conversion (note that the suppression effect has declined). An increase in the decay rate of the brief afterpotential following M to R conversion has been observed in 10 flies.

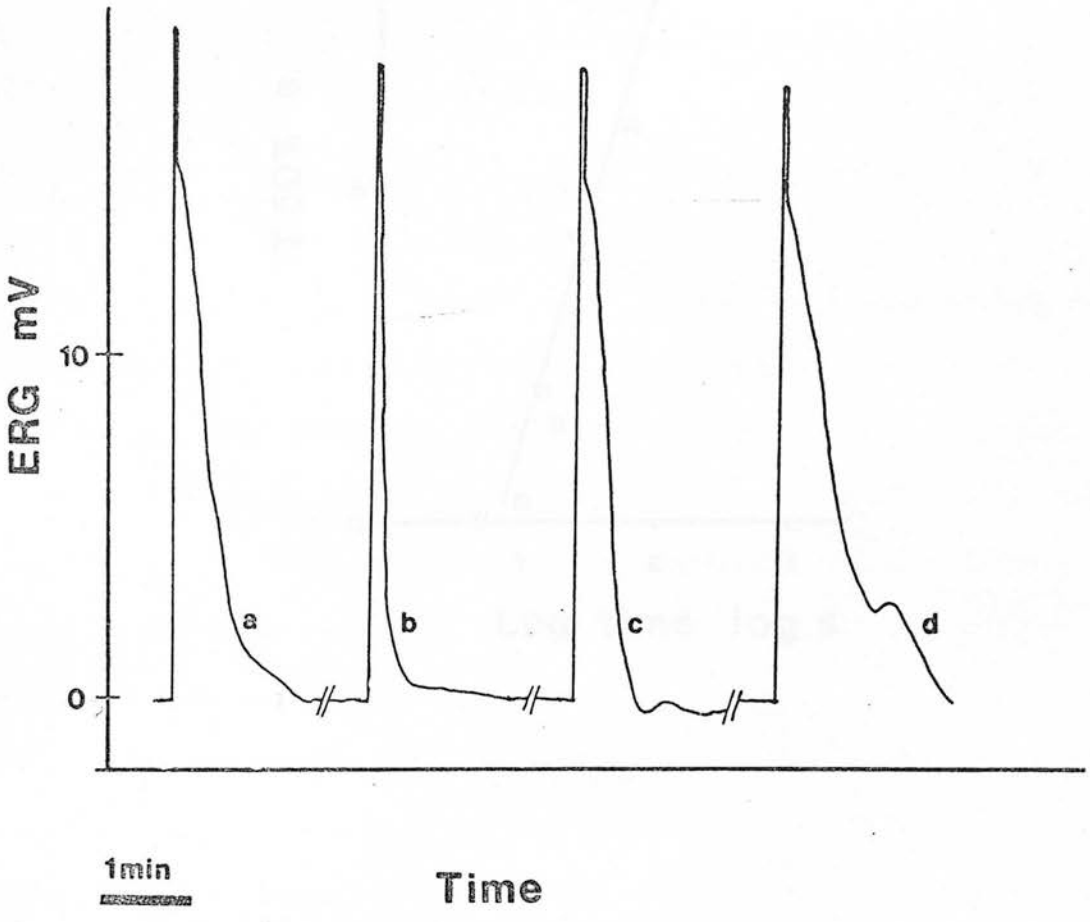
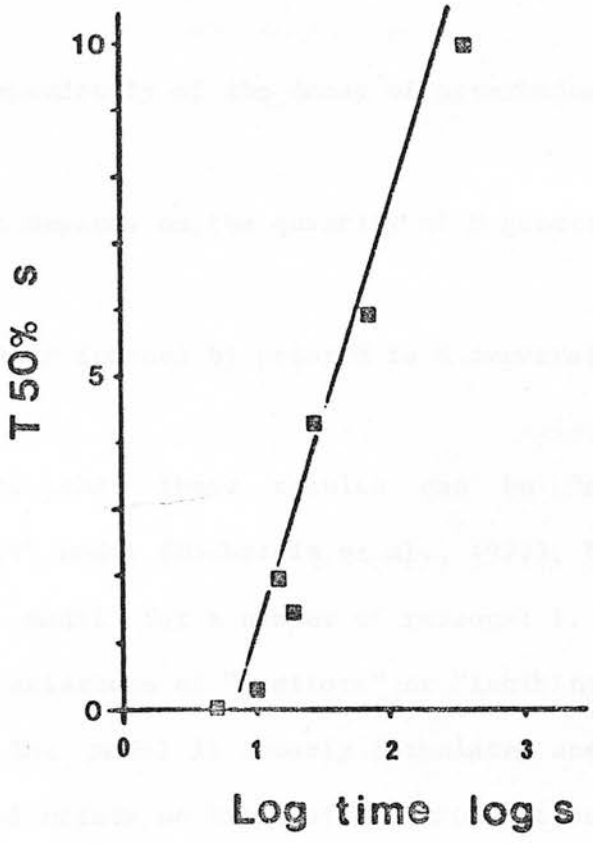


Fig. 3: This figure shows the correlation between the half-life of the brief afterpotential induced by a 1 s pulse from the adapting-flash and the logarithm of the time after M to R conversion ($r = .98$, $df = 5$, $P<0.01$).



seconds to minutes (Table 1; Fig. 1) to an asymptote that is (b) an intermediate level of the prolonged depolarising afterpotential which decays over a period of several hours (chapter 2). Such a dichotomous decay of afterpotential has not been described in any other species, but need not indicate different mechanisms. However, the behaviour of the brief afterpotential cannot be accounted for by the model of the prolonged afterpotential given in chapter 2. The Drosophila brief afterpotential resembles the prolonged afterpotential in Balanus and Limulus in three ways.

1. It decays independently of the decay of metarhodopsin.

2. Its decay time depends on the quantity of M generated.

3. Its decay time is reduced by prior M to R conversion.

Despite the fact that these results can be "explained" by the "excitor-inhibitor" model (Hochstein et al., 1973), I am reluctant to call upon this model for a number of reasons: 1. No corroborating evidence for the existence of "excitors" or "inhibitors" was found in chapter 2. 2. The model is loosely formulated and is difficult to test. 3. The model offers no biophysical explanation of events. A biophysical mechanism is suggested below.

R1-R6 in Drosophila are transiently hyperpolarised following long-wavelength illumination. This hyperpolarisation is particularly pronounced following M to R conversion (e.g. Fig. 1 chapter 2). Hyperpolarising afterpotentials in the photoreceptors of other species have been shown to be due to the activity of electrogenic pumps stimulated by the light induced influx of sodium (Balanus photorecep-

tor: Koike et al., 1971; Limulus photoreceptors: Brown and Lisman, 1972; Wulff and Mueler, 1973). It is reasonable to conclude that the hyperpolarisation of Drosophila R1-R6 is also due to the activity of an electrogenic pump. Experimental support for this inference is available. Electrogenic pumps are active mechanisms which are inhibited by metabolic poisons, oxygen starvation and cooling. The alternative mechanism for hyperpolarisation is a prolonged conductance change (e.g. to potassium as in the squid axon: see Kuffler and Nicholls, 1976). In the latter of these cases the hyperpolarisation is passive and relatively insensitive metabolic inhibition. Cosens (1971) has demonstated, by cooling, that an active mechanism contributes to the fast repolarising response of Drosophila photoreceptors. I propose that the suppression of the brief afterpotential in R1-R6 of Drosophila is due to temporary activation of an electrogenic pump.

Hamdorf and Schwemer (1975) have reported evidence that oxygen is required for the normal decay of the PDA in Calliphora photoreceptors. If the decay of a PDA is induced by a decline in membrane conductance (say, to sodium) the process should be passive. Hamdorf and Schwemer's data are consistent with active pumping of sodium ions. It seems possible that the brief afterpotential reported here in Drosophila photoreceptors displays kinetics controlled by an electrogenic pump, and that in this respect it resembles the PDA in other species. On this, speculative, interpretation of events, the prolonged afterpotentials in Drosophila would arise when the coupling ratio of the electrogenic pump is insufficient to counteract the metarhodopsin-correlated conductance. This model implies that the electrogenic pump is part of a control system which attempts to maintain the dark resting potential of R1-R6 at a constant level. The model outlined above is capable of accounting for (a) the sigmoid

relationship between the level of PCNA and the amount of light delivered, (b) the change in the slope of the afterpotential to response amplitude relationship after M to R conversion (Fig. 2, chapter 2: compare triangles with other symbols), (c) the suppression of the brief afterpotential. Direct biophysical evidence is required to test this hypothesis.

CHAPTER 5

RETINULA CELLS R1-R6 IN THE COMPOUND EYE OF WILDTYPE *DROSOPHILA* ARE BLUE-ADAPTED AND ORANGE-ADAPTED AS THEY ARE IN THE WHITE-EYED MUTANT

1: INTRODUCTION

Willmund and Fischbach (1977) have reported that the behaviour of wildtype Drosophila melanogaster in a situation where they may choose to spend time in light or darkness can be altered by previous illumination of the flies with blue light. This change in behaviour is graded according to the amount of blue light delivered but asymptotes at a level which is the same as the behaviour of flies in which R1-R6 are deficient (mutants ora and rdgB). In darkness the behavioural modification persists for several hours, but if the flies are kept in the experimental chamber (which is illuminated with yellow light) the behaviour gradually returns to that of non blue-adapted wildtype flies.

These results suggest that an afterpotential in R1-R6 of wildtype Drosophila might cause the behavioural modification. All of the recent work on the PDA in R1-R6 in Drosophila has been carried out on white-eyed mutants. The reason for this, which is rarely made clear in the literature, is that it has been difficult to demonstrate the PDA, or the in vivo photopigment conversions, in the wildtype (red eyed) fly. For instance using an illumination procedure identical to that used in chapter 2 I have found that even after 100 times the amount of light required to give a maximal PCNA in white-eyed Drosophila the afterpotential induced in wildtype flies is so small as to pass unnoticed except under the most careful scrutiny. An afterpotential was induced, and was removed by orange light but repeated experiments and statistical treatment were required to demonstrate the small effect. It must be assumed that other laboratories have experienced difficulty in demonstrating a PDA in R1-R6 of the wildtype since there are no reports in the literature.

The difficulty in demonstrating a PDA in R1-R6 of the wildtype is generally taken as evidence for the "function" of the red screening pigments in preventing the accumulation of metarhodopsin M580 and the consequent PDA in R1-R6 of the wildtype (Cosens and Briscoe, 1972; Cosens and Wright, 1975; Minke et al., 1975). However, it is clear that the screening pigment sheath in the secondary pigment cells of wildtype and cinnabar flies can not offer protection to retinula cells whose rhabdomeres are directly illuminated on their optical axis with blue light. However, the ommochromes in the retinula cells of wildtype may afford some protection even to the retinula cells of "on axis" rhabdomeres (longitudinal pupil, see Franceschini, 1975).

In order to account for the absence of evidence from the ERG of the PDA in R1-R6 it was necessary to assume that "on axis" retinula cells do experience a PDA but that the ERG is an insensitive measure of this event if only a few retinula cells are in PDA (say 50 out of 4,000). An alternative hypothesis is that the blue/orange adaptation observed in white-eyed mutants is not representative of the behaviour of retinula cells in the wildtype fly. For instance, it might be a pleiotropic effect of the mutations altering the screening pigments (w, bw, cn).

As before, the ERG was used in the present study as a tool to investigate the behaviour of R1-R6 (Cosens and Wright, 1975). This chapter demonstrates that when Drosophila are illuminated inside a table tennis ball, permitting direct illumination of most of the rhabdomeres of the eye, wildtype and cinnabar Drosophila can be blue/orange adaptated in a fashion similar to that observed in white-eyed flies. The only important difference between phenotypes is in the amount of light required to cause the adaptation. This finding

is consistent with leaking light laterally through the secondary screening pigment cells into adjacent ommatidia in the white-eyed fly with the result that more light reaches each rhabdomere. The results demonstrate that most of the light reaching the rhabdomeres of white-eyed flies is indirect. It is concluded that PDAs do arise in R1-R6 of wildtype flies that are directly illuminated by blue light and that the studies of white-eyed Drosophila have described events which are of physiological significance to the wildtype.

2: MATERIALS AND METHODS

The experiments were conducted in two parts at two differnent locations. An initial series of observations was made at Freiburg using the stocks and filters used by Willmund and Fischbach (1977). The experiments were later repeated at Edinburgh.

2.1: Experiments at Freiburg

Insofar as was compatible with making electrophysiological recordings from the eye of the fly an attempt was made to mimic the conditions of the behavioural experiments reported by Willmund and Fischbach (1977). The flies were from the same stocks and the colour filters were as used in the behavioural experiments. The experiments were carried out in an environmentally controlled room at 23 °C and 60% relative humidity.

The flies were Drosophila melanogaster; wildtype (red-eyed), cinnabar (bright red eyed) and white (white-eyed) flies were used. The flies were raised (on standard medium) in incubators at 23°C on a

12:12 L:D cycle.

Recording procedure: The flies were mounted on their sides in dental wax. ERGs were obtained from the flies using standard procedures. Glass microelectrodes filled with 3 molar KCl were inserted into the fly, one in the retinula cell region of the eye beneath the cornea and the second (reference) in the thorax. The electrodes were mounted directly into electrode holders and preamplifiers. Signals from the preamplifiers were fed to an electrometer amplifier (W-P. I. Model: M4-A; gain setting = 1) and the d.c. amplifier of a Tektronix 7504 oscilloscope; from there to a Perkin Elmer 56 penrecorder.

Illuminating most of the rhabdomeres of the eye: Traditionally electrophysiological preparations are illuminated with a directional light source. As a result only certain on-axis rhabdomeres can be directly illuminated; in an attempt to circumvent this problem the fly was mounted inside an illuminated sphere made from two table tennis balls (see Fig. 1). The sphere was illuminated from the outside using two Rollei slide projectors which were used together as the adapting light. The projectors were operated synchronously using the remote control facilities and the spectral composition of the light was controlled by 5 X 5 cm plexiglas colour filters (see Table 1). The projectors were fitted with heat filters and the temperature inside the ball was less than 1 C warmer than the room temperature after 1 hour of yellow illumination at 16 W m^{-2}.

A testflash of 0.125 seconds duration was delivered to the top of the sphere by an optical fibre, the light source was a third

Figure 1: A diagram of the illuminated sphere in which the fly was placed for the electrophysiological recordings. The sphere was constructed from two table tennis balls. The top ball was cut as shown to permit entry of the microelectrodes. No direct light could reach the preparation. E1 and E2: electrodes inserted into the eye and the thorax of the fly; W: the dental wax on which the fly was mounted.

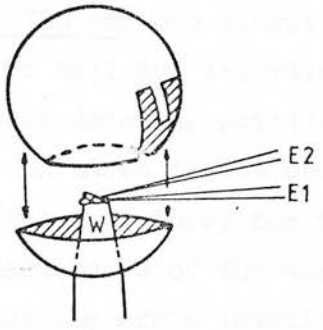


Table 1. Maximum light intensity measured inside the TT ball.

Röhm plexiglas filter number	colour of light	light intensity $W\ m^{-2}$
627[1]	blue	1.4
303[2]	yellow	16
-	white (testlight)	0.2

The light intensity (below 700 nm) was measured by cutting a new hole in the top table tennis ball and inserting the probe of a Kettering radiant power meter into the position occupied by the fly. Readings were taken in three directions; the variability measured was less than a factor of three for the adapting lights. The measurements are underestimates of the amount of light available to the fly because the probe interferes with internal reflections. The testlight intensity shown is with the probe directed upwards, with the probe facing laterally the intensity was about one tenth of the figure shown.

notes

1. bandpass filter: half-bandwidth 50 nm; max = 455 nm
2. long-wavelength pass filter, 520 nm is the half-transmission point

projector. The timing of the testlight was controlled manually using a photographic leaf shutter.

When a successful penetration of the eye gave a "typical" ERG response to the testlight the eyes were light adapted using various regimes to look for the effects of blue and yellow light. Details of the illumination procedures are given in the results section and in the figure legends.

Measurements: corneal-negative potentials are plotted upward on the figures. Two measurements were made from the records: the size of the response to the constant testflash, the response amplitude; and the ERG potential baseline in darkness, the afterpotential (see Fig. 2).

2.2: Experiments at Edinburgh

The methods used in part A were adapted for use with the recording equipment described in chapter 2.

The flies were wildtype and white Drosophila melanogaster with the same genetic background (Haren stocks).

The sphere was illuminated from 9.5 cm with the adapting light, and from 5 cm with the testlight. The testlight was set to maximum intensity to deliver a 250 ms flash at intervals of 4 s.

The light intensity inside the sphere was estimated by fixing a table tennis ball onto the probe of the digital photometer with a hole cut to allow the sensor to measure the light inside the ball. Measurements were made with the dummy sphere in the position of the real one. This gave readings of 1.4 W m^{-2} (3.4 X 10^{14} photons cm^{-2} s^{-1}) for the blue adapting light, 4.4 W m^{-2} (14.2 X 10^{14} photons

cm^{-2} s^{-1}) for the orange adapting light and 21 mW m^{-2} (5.2 X 10^{12} photons cm^{-2} s^{-1}) for the blue testflash. A difference in these values by a factor of less than two was obtained by rotating the dummy sphere. Balzers broadband colour filters were used (see chapter 2 for characteristics).

3: RESULTS

3.1:

The effects of illumination with blue and yellow light upon the ERG was examined in 5 wildtype, 2 cinnabar, and 1 white Drosophila melanogaster using the illuminated sphere constructed from table tennis balls (Fig. 1). Each fly gave results which satisfied at least one of the criteria for a PDA in R1-R6 listed below.

1. Following blue illumination a substantial reduction in the ERG response to a constant intensity testflash was measured which (a) was graded according to the amount of light delivered to the preparation (b) persisted for more than one minute after the blue light was switched off, (c) was of a magnitude not observed following yellow illumination and (d) was reversed by yellow light.

2. Accompanying the response size alterations a prolonged shift in the ERG dark-baseline or afterpotential was observed.

3. A clear negative correlation between reponse size and afterpotential was measured during adaptation with blue light or reversal with yellow light.

Taking each of these criteria as diagnostic of a PDA in R1-R6 all preparations gave evidence demonstrating that R1-R6 were adapted as in white-eyed Drosophila (chapter 2).

Fig. 2 is a photograph of a penwriter recording from one of the cinnabar preparations. It demonstrates the shift in the ERG baseline (afterpotential) following blue light. The reversal of this effect with yellow light is also shown. Figure 3 presents evidence suggesting that the progress of blue-adaptation is governed by the amount of blue light in the stimulus, a result which is consistent with findings in the white eyed mutant (chapter 2).

In all of the flies investigated 10 s of yellow light at 16 W m^{-2} was sufficient to bring about 90% reversal of the effects of blue light. Figure 4 shows the reversal of the afterpotential shifts by yellow light at 1.6 W m^{-2}. The halftime for reversion under these conditions was about 25 seconds. In contrast only a very small decline in afterpotential was observed during 3.5 hours of darkness following full blue adaptation. Yellow light restored the afterpotential and the response amplitude to the dark-adapted level as in figure 2. These results, while qualitative, are consistent with the behaviour of R1-R6 in white-eyed Drosophila (chapter 2) and indicate that the rate-constant for the dark reversion of metarhodopsin M580 to rhosdopsin R480 is the same in cinnabar as in white-eyed Drosophila (see chapter 2).

The correlation between response amplitude and afterpotential is demonstrated in Figure 5. Figure 5a shows this correlation ($r = -0.985$, $df = 11$, $P<0.01$) in data from an experiment lasting 15 minutes using the wildtype. Figure 5b shows the correlation obtained ($r = -0.90$, $df = 22$, $P<0.01$) from a cinnabar preparation; the points

Figure 2: A penwriter recording demonstrating the prolonged afterpotential in the ERG of a cinnabar Drosophila. After 16 minutes of blue light (0.14 W m^{-2}) a substantial shift in the dark-level of the ERG persists for at least 1 minute in darkness (PCNA). The circles indicate that a testflash was delivered. Ten second doses of yellow light (1.6 W m^{-2}) restore the ERG baseline and the response amplitude to the dark-adapted level. Larger doses of yellow light (1 min and 2 min are shown) are followed by afterpotentials but never of the magnitude observed after blue light. The zero of the ERG (mV) scale is the mean of ten measurements made 1 min after yellow adaptation during 6 hours of experiments. The figure illustrates how response amplitude (ra) and afterpotential (Va) were measured from the original recordings. This figure should be compared with the similar Figure 1 in chapter 2 which shows a record from a white-eyed fly. The source of the periodic noise, which should not be confused with the response amplitude, is unclear.

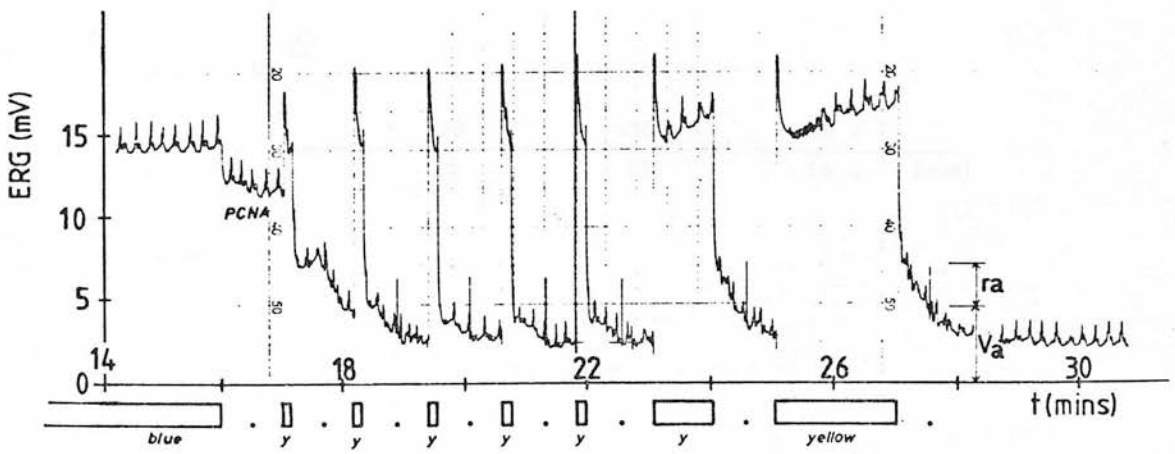


Figure 3: The afterpotential one minute after adaptation is plotted against the duration of exposure to blue light. Each point is an independent measurement from a cinnabar fly. The experiments were carried out over a six hour period: d.c. potentials were measured from the same reference potential throughout the experiments. Circles: adaptation with blue light at 1.4 W m^{-2}; squares: adaptation with blue light at 0.14 W m^{-2}. The points fall on a similar curve suggesting that the amount of adaptation is controlled by the amount of blue light delivered.

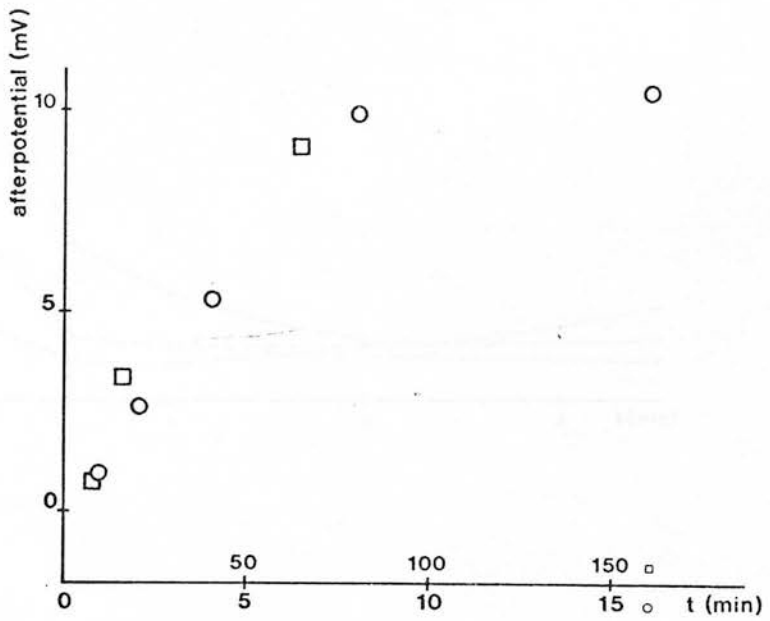


Figure 4: The reversal of the effects of blue light shown by squares in Figure 3. Yellow light at 1.6 W m^{-2} restores the afterpotential to its original level. The afterpotential was measured 1 min after yellow adaptation.

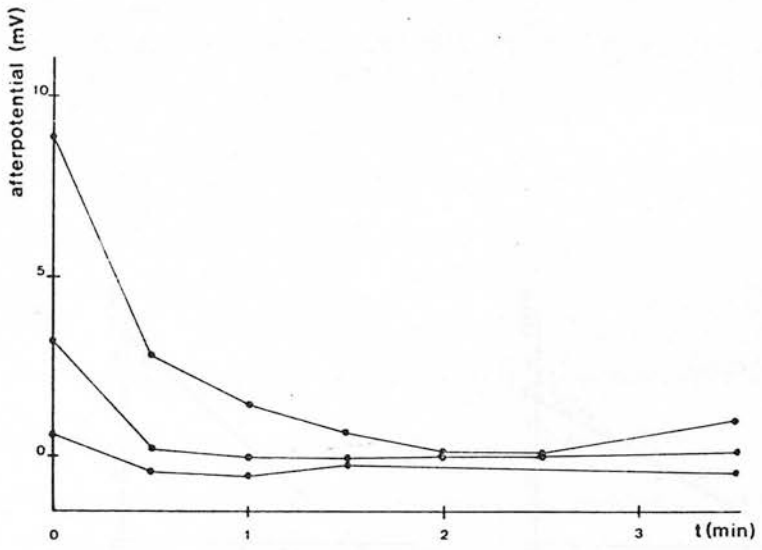
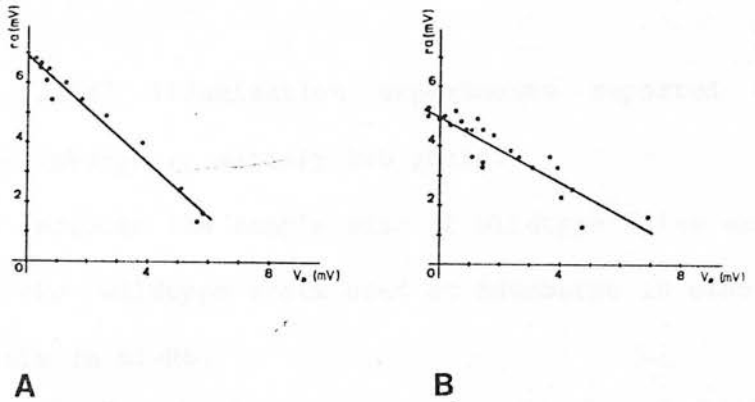


Figure 5: Examples of correlations between response amplitude to a fixed intensity testflash (ra) and the afterpotential (Va). Fig. 5a shows data from a wildtype fly; the zero on the afterpotential scale is arbitrary ($r = -0.985$, $df = 11$, $P<0.01$, slope $= -0.91 + 0.049$ (S.E.)). Fig. 5b shows data from the cinnabar fly of Figs. 2, 3 and 4; the data were collected systematically during experiments over a six hour period ($r = -0.904$, $df = 22$, $P<0.01$, slope $= -0.55 + 0.056$ (S.E.)). The data for both correlations were obtained thirty seconds after small doses of yellow light at 1.6 W m^{-2} which reversed prior blue-adaptation.



A B

plotted were taken systematically during a series of experiments lasting 6 hours. The zero on the afterpotential co-ordinate is the mean of ten measurements of the afterpotential level made one minute after complete yellow adaptation as it is in Figures 2, 3 and 4. The unusual d.c. stability of this preparation permitted this demonstration of the afterpotential effect over long time periods: previously reported correlations were obtained during much shorter experiments.

The difference in the slope of the regression between the wildtype and the cinnabar fly is in part due to differences in the dark-adapted response amplitude (e.g. see chapter 2), but activation of the "pupil mechanism" in the wildtype during the PDA may also have contributed.

3.2:

The "whole field" illumination experiments reported above were repeated at Edinburgh to satisfy two goals.

1. To increase the sample size of wildtype flies examined and check that the wildtype stock used at Edinburgh is also subject to afterpotentials in R1-R6.

2. To counter the possible objection to one of the criteria for an afterpotential in R1-R6 used in part 3.1. The first of the criteria used there, it may be objected, is not unequivocal evidence for an afterpotential in R1-R6 because there are three other mechanisms which could give rise to it. (a) Spectral adaptation alters the amount of rhodopsin present in R1-R6 and this may be expected to alter the number of photons captured by the visual pigment and result in the response amplitude changes reported (e.g. see Hamdorf and Schwemer, 1975). (b) The presence of large quantities

of metarhodopsin may directly suppress the activity of rhodopsin thus producing the changes observed (e.g. the argument of Razmjoo and Hamdorf, 1976, which is examined in chapter 3). (c) The changes may be due to the activation of the "pupil mechanism" in the wildtype (see Franceschini, 1975; Stavenga, 1976). For this reason the second criterion, a measured shift in the afterpotential, is closely checked in this section. The regime used inevitable concentrates on the effects of blue light upon the response amplitude. This is because the d.c. stability of the preparations over the long adaptation periods used was often poor (judged by the variability in the ERG baseline after each of the periods of orange adaptation).

Sixty one experiments were performed on 10 wildtype flies. The flies were subjected to doses of light of various intensities for periods of 1 to 45 minutes. After each dose the fly was kept in darkness (except for the testlight which flashed continuously during the experiments) for two minutes. At this time the response amplitude and ERG baseline were compared with their respective values immediately before adaptation. During the two minute dark period the response amplitude increased in size (dark adaptation and recovery of brief afterpotential, chapter 4) but was within a few percent of its asymptote by the end of the two minute period. At the end of this period a 10 s pulse of orange light was applied, followed by another two minutes of darkness at the end of which the response amplitude and ERG baseline were compared with their respective values immediately before the orange illumination.

Each experimental sequence was separated by a 1 minute pulse of orange and a 2 minute dark period.

Fifty three of 61 experiments (Chi-square = 33.2, df = 1, $P<0.005$) in 10 out of 10 wildtype flies showed a clear reduction in

response amplitude two minutes after blue-adaptation. In all 53 of these cases the response amplitude then increased after orange-adaptation. In all of the flies in which an experiment gave a negative result a positive result was obtained when a larger dose of blue light was delivered. Thus no preparation failed to satisfy the response amplitude criteria of blue/orange adaptation in R1-R6

Forty three of 61 experiments (Chi-square = 10.25, df = 1, $P<0.005$) in 7 out of 10 wildtype flies resulted in an increase in the ERG baseline following blue illumination and a clear reduction following the subsequent orange illumination.

The three wildtype flies in which the afterpotential shift was not evident were judged to be noisy and d.c. unstable by independent criteria. In the wildtype flies which gave d.c. stable recordings the evidence for the ERG afterpotential shifts was clear cut (Fig. 6).

These results establish that a depolarising afterpotential follows blue light in wildtype Drosophila. This is not consistent with any of the alternative explanations of blue/orange adaptation in wildtype Drosophila listed above.

Four wildtype and 4 white-eyed Drosophila were adapted in the illuminated sphere in order to compare the amount of blue light required to produce the adaptational changes.

In the wildtype the change in response amplitude elicited by blue light is an asymptotic function of the amount of blue light delivered (Fig. 7). Table 2 reports the correlation coefficients and rate constants obtained by fitting the data to an equation of the form $y = A + B\, e^{-Rx}$, where x is the duration of illumination with blue light and R is the rate constant for adaptation. The equation is a good fit. The rate constants are quite variable between flies.

Figure 6: A penwriter record demonstrating a partial PCNA in a wildtype Drosophila.

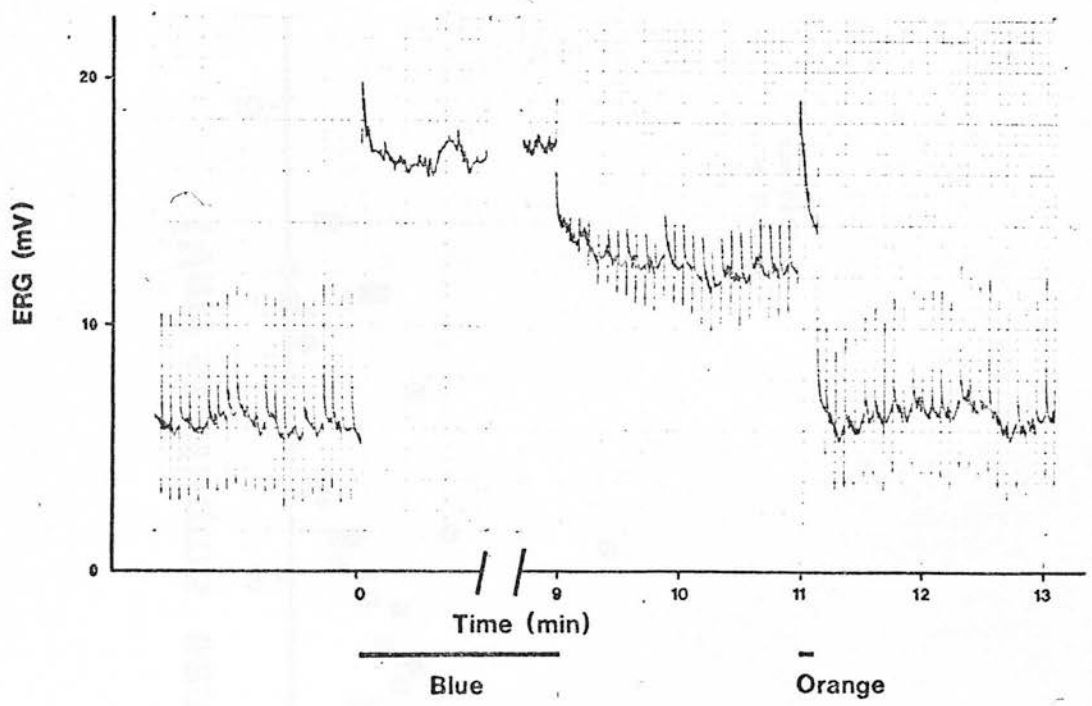


Figure 7: The figure shows the progressive reduction in the response amplitude to a fixed intensity stimulus after exposure to blue light. Data obtained from four wildtype Drosophila is plotted on the figure.

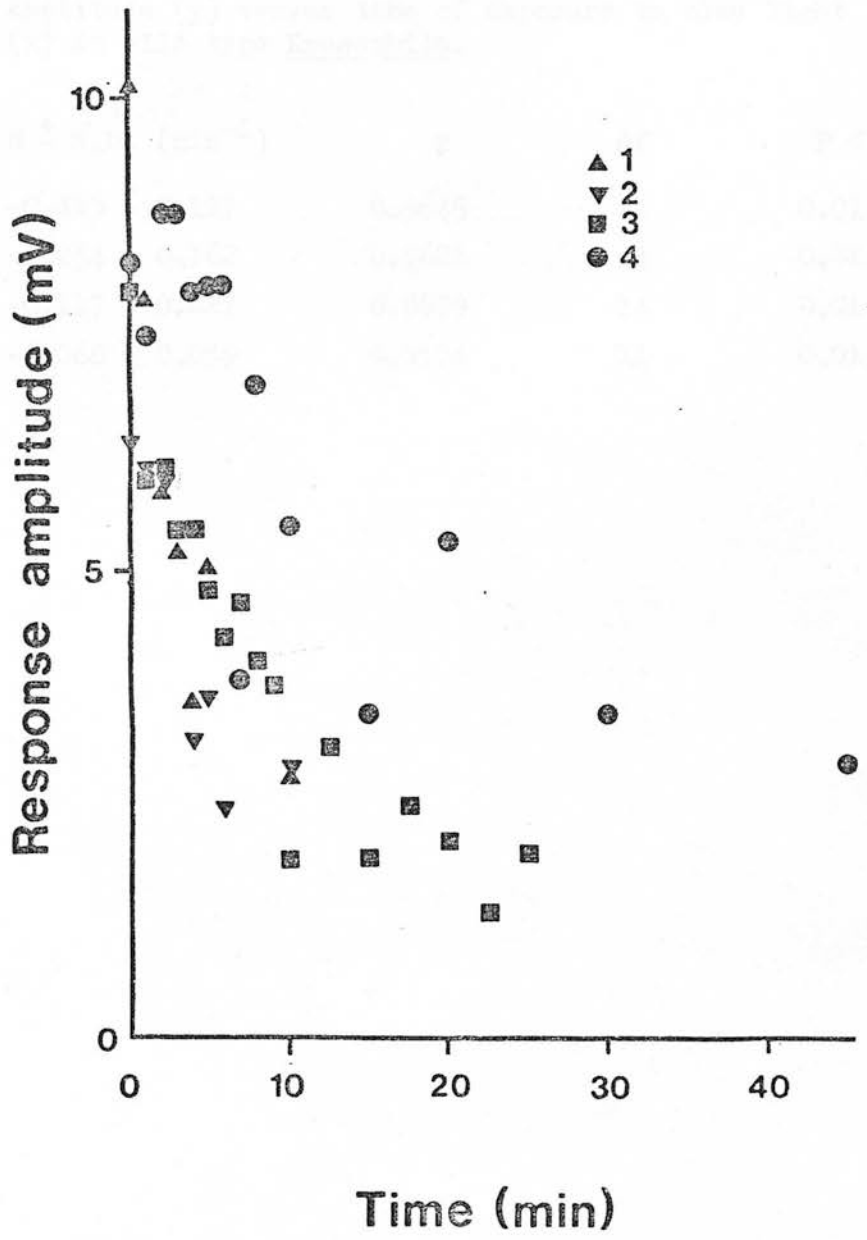


Table 2. The rate constant, R, and correlation coefficients for the equation $y = A + B e^{Rx}$ fitted to the response amplitude (y) versus time of exposure to blue light (x) in wild type Drosophila.

	$R \pm$ S.D. (min^{-1})	r	df	$P <$
wild 1	-0.415 ± 0.127	0.9645	4	0.01
wild 2	-0.234 ± 0.162	0.9622	4	0.01
wild 3	-0.117 ± 0.027	0.9639	14	0.01
wild 4	-0.068 ± 0.039	0.8574	11	0.01

Figure 8: As figure 7 but showing data from white-eyed flies.

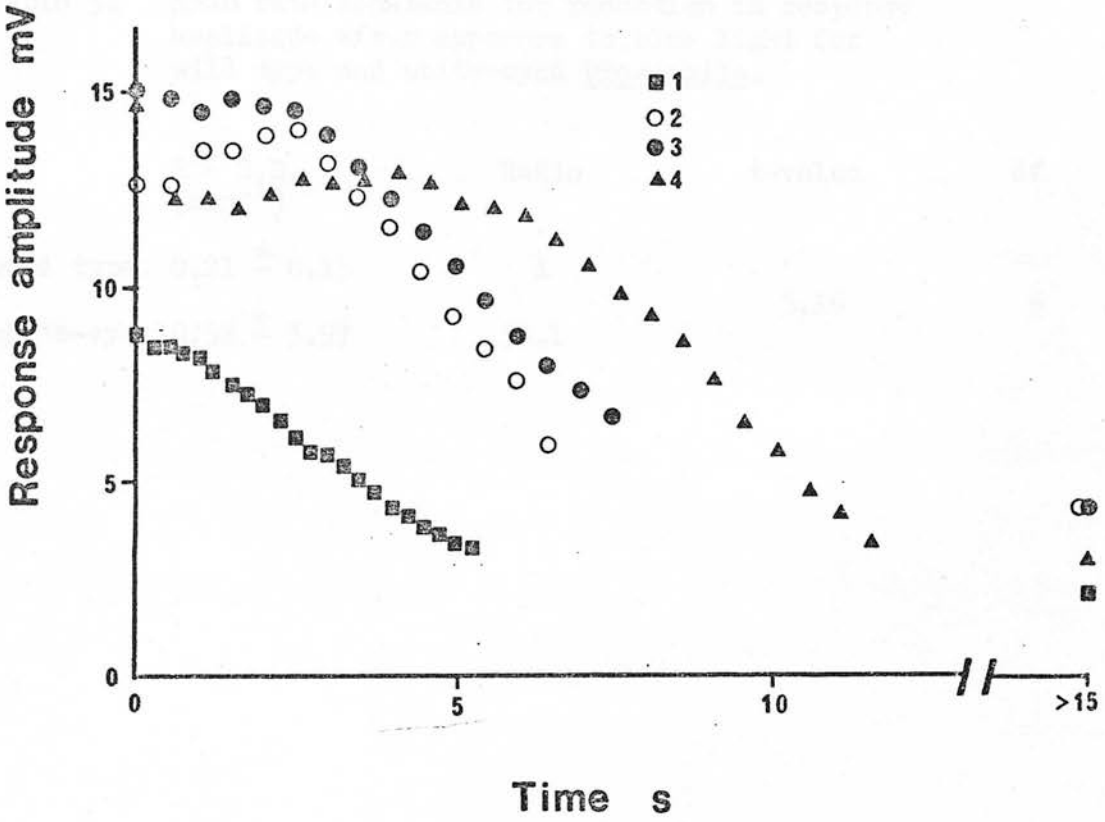


Table 3. Mean rate constants for reduction in response amplitude after exposure to blue light for wild type and white-eyed *Drosophila*.

	$R \pm S.D.$ (min^{-1})	Ratio	t-value	df	$P <$
wild type	0.21 ± 0.15	1	5.16	6	0.005
white-eye	10.52 ± 3.97	50.1			

Table 3 shows the mean. In the white-eyed flies the response amplitude was a sigmoid function of the amount of blue light delivered as reported in chapter 4 (Fig. 8). The explanation of this difference in functional response probably lies in the fact that the wildtype eyes were not evenly illuminated so that some cells were blue-adapted more rapidly than others. The dose of light required for 63% reduction in the response amplitude from the white-eyed flies was estimated from the plotted data giving the mean value for the rate constant given in Table 3.

The analysis indicates that using the set-up at Edinburgh the wildtype requires 50 times more light for blue-adaptation than the white-eyed fly even under "whole field" illumination ($t = 5.16$, $df = 6$, $P<0.005$, t test on rate constants). It is obvious that even under "whole field" illumination, only a small fraction of the light exciting individual retinula cells R1-R6 enters through the physiologically relevant dioptric apparatus of that cell.

4: DISCUSSION

The results clearly demonstrate that wildtype Drosophila can be blue adapted and orange adapted in the same way as white-eyed flies. It is evident that long term changes in the ERG baseline are induced by the spectral adaptation used. I conclude, following the arguments given in chapter 2 for white-eyed Drosophila, that wildtype (and cinnabar) Drosophila experience long lasting changes in the resting potential across the membranes of retinula cells R1-R6 in the retina, and that the changes in resting potential are controlled by the amount of metarhodopsin remaining after visual transduction. The changed resting potential, or afterpotential, in each of these cells is thus

governed by the spectral history of that cell.

Two differences were noted between the genotypes. 1. The shape of the relationship between the induced adaptation and the amount of blue light delivered is different. 2. The amount of light required to effect the adaptation is larger in wildtype flies. The first of these is probably due to heterogeneities in the illumination of retinula cells in the wildtype. The latter may represent a genetic difference between the flies but is more likely due to the absorption of light by the screening pigments of the wildtype. There is every reason to conclude that the blue/orange adaptation described in the white-eyed mutant occurs in wildtype flies and that the white-eyed flies provide an adaquate model of the visual process in the wildtype.

The behavioural results of Willmund and Fischbach (1977) are clearly largely explained by a prologed afterpotential in R1-R6 and the consequent reduction in the response amplitude from these cells. This chapter thus demonstrates that the behavioural modification reported by Willmund and Fischbach (1977) is a behavioural consequence of changes in the resting potential of primary sensory cells. Attention is thus focussed on peripheral processing of optical information and the behavioural importance of changes in resting potential (Mendelson, 1971).

CHAPTER 6

A CAPACITANCE MODEL OF THE EXTRACELLULAR POTENTIAL IN AN ELECTRICALLY ISOLATED COMPARTMENT

1: Introduction

Electrical recordings of the sustained changes in potential associated with blue/orange adaptation in R1-R6 of Drosophila are of opposite polarity but essentially similar in form whether recorded with extracellular or intracellular electrodes (e.g. see chapter 2 and Minke et al., 1975). Because of this, and the fact that the transmembrane potential of R1-R6 is equal to the intracellular potential minus the extracellular potential, these three potentials must be of similar form. This inference is supported by transmembrane recordings from the dronefly Eristalis (Tsukahara et al., 1977), and appears to contrast with results from other species (Sepiola: Pynsent and Duncan, 1977). The reason for this difference may be a peculiarity of the anatomy of insect visual systems, and the way in which the ERG is recorded. The ERG of insects (and often the intracellular potential, e.g. Minke et al., 1975) is recorded relative to a remote indifferent electrode in the haemolymph. This electrode is often placed in the thorax or in the proboscis. It has been known for some years (e.g. Burtt et al., 1965) that standing d.c. potentials exist within the insect optic lobe and retina and between these structures and the general haemolymph. These d.c. potential differences imply the existence of high resistance barriers ("barrier membranes") which effectively isolate these parts of the nervous system from the general haemolymph, and define compartments within the eye.

Heisenberg (1971) has applied the "barrier membrane" concept to Drosophila. He suggested, from electrical measurements, that at least two barriers exist in this species, one separating the peripheral retina from the lamina in a position similar to a basement membrane, and the second separating the lamina from the haemolymph.

Shaw (1977) has measured the electrical resistance between similar putative extracellular compartments in the locust. These measurements and observed restrictions on the exchange of dye markers between these areas strongly support the compartment hypothesis.

This chapter examines the behaviour of neurons inside an ideal compartment. That is, one which is completely isolated from the point of potential reference. Under these circumstances the various "resistive" models of extracellular potentials are insoluble (e.g. Duncan and Croghan, 1973; Shaw, 1975). The model is thus interesting theoretically because it accounts for extracellular potentials in the absence of current flow through an extracellular resistor. The model relies on the concept of capacitance.

2: Results

2.1: The Model

Consider a group of, say, j neurons and their shared extracellular space to be contained within an electroneutral isolated compartment. Further consider that at equilibrium the extracellular space is isopotential and the inside of each neuron is isopotential. The model thus relates to an idealised compartment which does not exchange charge with its surroundings. That is, the resistance between the compartment and the remainder of space is infinite. The model also assumes that the transmembrane potential of each cell within the compartment is uniform over its entire surface. The potentials within this compartment will be taken as the asolute potentials represented here as potentials to ground: V0 and Vi. The charge within each cell and within the extracellular space are represented by qi and q0. C0

and Ci represent the capacitances within the compartment to ground and C = q/V by definition.

The model is intended to correspond to the compartment of the peripheral retina of Drosophila, and ground is taken to represent the location of the remote indifferent electrode in the body haemolymph. The necessary assumption of the model is that no exchange of charge occurs between the compartment and the remainder of the animal. The results, however, should apply when only very small exchanges of charge have occurred, for instance, when only a small time has elapsed since an exchange of charge across cell membranes within the compartment.

Solutions are provided for the relationship between intracellular and extracellular potentials and the transmembrane potentials in two cases. 1: A general solution. 2: A special case solution when it is assumed that all cells have equal capacitances.

2.2: Solutions

2.21: General case

The extracellular potential within an isolated electroneutral compartment is a simple function of the intracellular potentials of each cell within the compartment and the capacitances of these cells:

$$V0 = -\Sigma(Vi.Ci)/C0 \qquad \text{(eqn. A4: see appendix)}$$

The transmembrane potential of each cell is Vi0 = Vi - V0 from which can be obtained the relationship between the extracellular potential and the transmembrane potentials of cells within the compartment:

$$V0 = - \Sigma(Ci.Vi0)/(C0.(1 + \Sigma(Ci/C0)) \qquad \text{(eqn. A8)}$$

2.22: Special case solution

A more tractable solution is obtained if it is assumed that all of the cells within the compartment have equal values of capacitance to ground. In this case the extracellular potential is directly proportional to the mean transmembrane potential of cells within the compartment:

$$V0 = - r.\bar{V}i0 \qquad \text{(eqn. A12)}$$

where r = CE/C0 and CE is the capacitance of an equivalent lumped intra- to extracellular space:

$$CE = (nCi.C0)/(nCi + C0)$$

The extracellular potential is related to the mean intracellular potential according to the equation:

$$V0 = -\bar{V}i.(nCi/C0) \qquad \text{(eqn. A14)}$$

3: Discussion

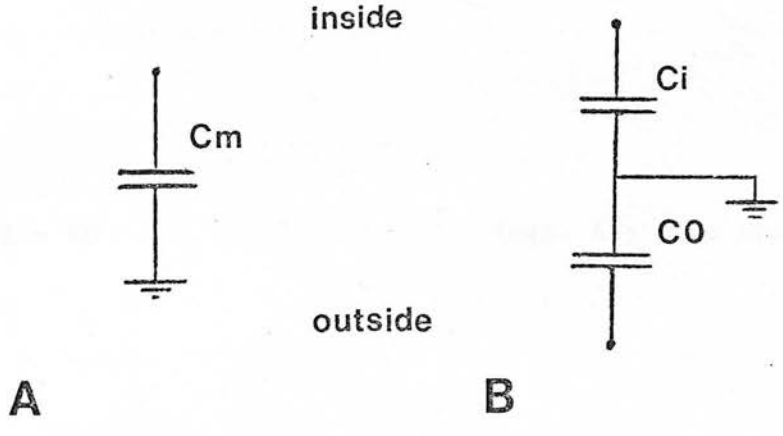
The model predicts that the extracellular potential recorded in an ideal compartment is directly proportional to the mean intracellular potential of cells within it, and is of opposite sign; and that both extracellular and intracellular potentials are directly proportional to

the transmembrane potential of cells within the compartment. Simultaneous intracelluar and extracellular recordings are required to test these predictions rigorously, but as noted in chapter 2 the available evidence is in accord with these predictions.

The use of cell capacitance to predict standing d.c. potentials appears to be novel and stands in contrast to models which rely on current flow through extracellular resistors (e.g. Shaw, 1975; Duncan and Croghan, 1973 ; see also Rodiek, 1973). It remains to be determined experimentally what contribution cell capacitance actually makes to the distribution of d.c. potentials: the result will depend upon how closely the real compartments within the nervous system correspond to the ideal presented here. However, as noted, the cell capacitance may contribute to the kinetics of intracellular and extracellular responses because at very short time intervals after a redistribution of charge across cell membranes within the compartment the compartment approximates to ideal (very small exchange of charge across the compartment boundary). The present analysis suggests that an analysis of the kinetics of intra and extracelluar potentials may gain from considering the membrane capacitance to be in series with the capacitance of the extracellular space to ground (see Fig. 1).

However, it must be noted that the peripheral retina of Drosophila cannot be an ideal compartment. For instance, the axons of the photoreceptor cells pass out of the retinal compartment into the lamina compartment where the extracellular space is at a different potential (Heisenberg, 1971). These axons cannot be isopotential and maintain a uniform transmembrane potential, so there must be a flow of intracellular current. The idealised model described above fails to account for the presence of lamina-origin transients in the ERG. A

Figure 1: The usual representation of the capacitance of a neuron is shown in A. Here the membrane capacitance, Cm, is taken to represent the capacitance between the intracellular space of the neuron and the extracellular space surrounding it. This representation holds true if the extracellular space is grounded as in A. When the extracellular space is not grounded, as in B, and lies in an ideal compartment the capacitance across the cell membrane consists of two components in series, Ci the capacitance of the intracellular space to ground and CO the capacitance of the extracellular space to ground. Ci corresponds to Cm. The capacitance of the extracellular space within a compartment will clearly alter the behaviour of the neuron. For instance, the smaller is the value of CO the less is the charge that need be transferred across the membrane to effect a given change in resting potential.



more detailed knowledge of the pathways of charge exchange between extracellular compartments is required.

A marriage of the capacitance model described here and the resistive model of Shaw (1975) is briefly discussed in the concluding chapter.

4: Appendix

$V_i = q_i/C_i$ (eqn. A1; from the definition of capacitance)

$q_0 = -\Sigma q_i$ (eqn. A2; from electroneutrality)

Therefore

$V_0 = -\Sigma q_i/C_0$ (eqn. A3; $V_0 = q_0/C_0$)

and

$V_0 = -\Sigma(V_i.C_i)/C_0$ (eqn. A4; $q_i = V_i.C_i$ from eqn. A1)

Now

$V_{i0} = V_i - V_0$ (eqn. A5; from the loop theorem)

and

$V_{i0} = q_i/C_i - q_0/C_0$

therefore

$$Ci.Vi0 = qi - q0.Ci/C0$$

$$\Sigma Ci.Vi0 = \Sigma qi - q0.\Sigma(Ci/C0) \qquad \text{(eqn. A6)}$$

and

$$-\Sigma Ci.Vi0 = q0 + q0.\Sigma Ci/C0 \qquad \text{(substituting for qi from eqn. 2)}$$

$$= q0(1 + \Sigma Ci/C0)$$

therefore

$$q0 = -(\Sigma Ci.Vi0)/(1 + \Sigma Ci/C0) \qquad \text{(eqn. A7)}$$

This is the general solution for the charge in the extracellular space of an ideal compartment. From it, the general solution for the extracellular potential is

$$V0 = -(\Sigma Ci.Vi0)/(C0.(1 + \Sigma Ci/C0)) \qquad \text{(eqn. A8)}$$

In the special case when all Ci values are identical equation A7 simplifies to

$$q0 = -(Ci.\Sigma Vi0)/(1 + nCi/C0) \qquad \text{(eqn. A9; for n neurons)}$$

This equation simplifies to

$$q0 = -\Sigma Vi0.Ci.C0/(nCi + C0) \qquad \text{(eqn. A10)}$$

or

$q0 = - CE.\bar{V}i0$ (eqn. A11; $CE = (nCi.C0)/(nCi + C0)$; $\bar{V}i0 = \Sigma Vi0/n$)

Since $q0 = -\Sigma qi$, CE is the capacitance of the lumped intra- to extracellular space. From equation A11, and putting $CE/C0 = r$, which will be a parameter characteristic of each compartment system, the potential of the extracellular space becomes

$V0 = -r.\bar{V}i0$ (eqn. A12)

r, the compartment parameter, is equal to the ratio - V0/Vi0 and its value is determined by the ratio C0/nCi. Its equation is

$r = nCi/(nCi + C0)$ (eqn. A13)

which is an equation of the form $y = 1/(1 + x)$, where $x = C0/nCi$. This function is constrained between zero and one; and the magnitude of r, and hence the extracellular potential, declines as a sigmoid function of the logarithm of the ratio C0/nCi.

In the special case when all Ci values are identical, equation A4, which relates the extracellular potential to the intracellular potential, simplifies to

$V0 = - \bar{V}i.nCi/C0$ (eqn. A14)

CHAPTER 7

CONCLUSIONS

The goals of the thesis which were formally set out in chapter 2 (section 7) have been largely realised as reported in subsequent chapters.

The prolonged depolarising afterpotential in the peripheral retina of Drosophila was described in some detail in chapters 2 and 4, and the data presented there support the assertion that the d.c. level of the ERG potential reflects d.c. changes in the resting potential of photoreceptor cells, principally R1-R6. The ERG data indicate that the afterpotential in R1-R6 of Drosophila differs from the afterpotential reported in other species (Balanus, Limulus and Calliphora), but this need not indicate that different mechanisms are at work. For example, it is possible that the balance of the interaction between contributory mechanisms is different in different species.

A biophysical explanation of the prolonged depolarising afterpotential in Drosophila was advanced in chapter 2. According to this model there is a component of membrane conductance in R1-R6 which is correlated with the amount of metarhodopsin M580. Using this model, the afterpotential was incorporated into mathematical equations which describe the input-output behaviour of sensory receptors (Lipetz, 1969, 1971). A number of features of the behaviour of R1-R6, inferred from ERG recordings, are predicted by the modified equations (chapters 2 and 3). The modification of the equations of Lipetz is similar to that used by Laughlin (1975) to account for light-adaptation, so the new results reported in chapters 2 and 3 should apply equally to light-adaptation.

The model predicted, and experimental data showed, a negative correlation between the ERG d.c. potential (afterpotential) and the response amplitude elicited by a constant stimulus. This correlation

is due to ambient light and/or the M580-correlated conductance. It was suggested in chapter 2 that the afterpotential is a primary determinant of the response from R1-R6 in Drosophila and consequently has important consequences in the assessment of "sensitivity" (see chapter 3). This last result can be expected to be generally applicable. If this is true, and the modified equations used in chapter 2 can describe the behaviour of other sensory receptors, it would seem wise to categorise, and describe, adaptation in terms of the parameters of these equations (Vmax, Va, sigma and n). This suggestion is supported by the mathematical analysis of data from Calliphora photoreceptors. The analysis of the data of Razmjoo and Hamdorf (1976) in chapter 3 showed that there were adaptational effects upon Vmax and n after blue-adaptation, in addition to the originally reported effect upon sigma. Since the recordings were not genuine d.c. recordings, changes in Vmax cannot be distinguished from changes in Va. However, the Calliphora data is consistent with changes in Va associated with the presence of metarhodopsin. It should be interesting to determine the contribution of afterpotentials to the spectral adaptation in Calliphora and other species (e.g. Ascalaphus, Deilephila, Eledone: Hamdorf et al., 1972, 1973).

We know from the extensive work of Franceschini and Stavenga that the retinula cells in the eyes of many insect species contain pigment granules which migrate upon illumination giving rise to a system of sensitivity control analogous to the vertebrate pupil and hence known as the longitudinal pupil. Stavenga has shown that there is a close link between the migration of these pigment granules and the photopigment composition (review of Drosophila data in Franceschini, 1975; see Stavenga and Kuiper, 1977, Stavenga et al., 1977). In

chapter 5 it was shown that the depolarising afterpotential, well known in white-eyed Drosophila, also occurs in the red-eyed wildtype, and it is reasonable to infer that the afterpotential observed in R1-R6 of chalky Calliphora (Muijser et al., 1975) also occurs in the wildtype fly under experimental conditions. In nature, though, the red screening pigments in the secondary pigment cells and the longitudinal pupil of the retinula cells must oppose the accumulation of metarhodopsin and the consequent PDA in wildtype flies. However, this protection may not be complete. According to Hamdorf et al. (1973), 50% of the visual pigment of Calliphora will be metarhodopsin under daylight illumination. A similar figure may well apply in Drosophila because the pigment systems of the two flies are similar. Indeed in Drosophila a slightly higher figure for the metarhodopsin fraction may apply because dark regeneration is slower (see chapter 2, contrast Stavenga, 1975). Such a high value for the metarhodopsin fraction in nature is intriguing because, if true, it implies that afterpotentials are always present in daylight, that their magnitude will vary with the spectral composition of daylight, and hence that the behaviour of the photoreceptors alters throughout the day (note that the value available is based on calculations not on measurement).

The spectral composition of daylight varies through the day. For example at sunrise and sunset the blue/red ratio of daylight changes (Johnson et al., 1967). Furthermore, the spectral composition of light in nature also depends upon location; for instance beneath green leaves the blue component of daylight is enhanced with respect to the red (Gates, 1965 cited in Ringo, 1977). Ringo argues that the spectral dependence of lek behaviour (a courtship ritual) in male Drosophila grimshawi may have evolved to match changes in the

spectral composition of daylight. It is tempting to speculate on the mechanisms of such spectral preference. This is particularly true because of the demonstration in chapter 5 of the basis of changes in behaviour observed in the laboratory (Willmund and Fischbach, 1977).

It seems to me that there must be some function for the afterpotential because all of its known effects appear to disadvantageous yet these could be minimised by increasing the dark reversion rate of metarhosopsin to rhodopsin.

Attention was drawn in chapters 2 and 4 to the hyperpolarisation which follows illumination of Drosophila photoreceptors, particularly after M to R conversion. This transient hyperpolarisation and the brief and prolonged depolarising afterpotentials are clearly antagonistic. It was suggested in chapter 4 that the hyperpolarisation was due to the activity of an electrogenic pump. The same suggestion, and a particularly elegant demonstration of the antagonism of hyperpolarising and depolarising afterpotentials is given by Tsukahara et al. (1977) who describe the PDA phenomenon in the dronefly Eristalis. Like myself, these authors are sceptical of the value of the "excitor-inhibitior" model in the interpretation of the PDA. However, they skirt the issue of an alternative mechanism.

The "M580-conductance" model advanced in chapter 2 has been described adaquately there. As indicated in chapter 4 the "brief afterpotential" does not fit neatly into this model. The brief afterpotential has some features in common with the stimulus-coincident or fast-repolarising response identified in Figure 11 of chapter 2, and it remains to comment on this response. Tsukahara et al. (1977) explain the difference in the spectral sensitivity of this response and the slow repolarising response (the PDA).

The M580-conductance is clearly too small, and it decays too slowly, to make a major contribution to the stimulus-coincident conductance change. But if a similar visual pigment correlated conductance arises prior to the appearance of M580, and decays rapidly, the following model may apply: Upon isomerisation R480 is converted to a state M′ which induces a high conductance state at the membrane as long as it is present. M′ is either a transient intermediate between R480 and M580 or is freshly formed M580. M′ rapidly decays to M580. According to Stavenga (1976), Calliphora metarhodopsin is formed within 30 ms of the irradiation of rhodopsin, but, of course, the speculative M′ may be spectrally identical to M580.

The suggested mechanism is not inherently improbable. Fesenko and Lyubarskiy (1977) have incorporated fragments of rod outer segments into bi-layer lipid membranes. They observed a light induced increase in membrane current (which in their experimental system corresponds to a light induced increase in membrane conductance) which occured milliseconds after a flash of white light, and which had a lifetime of a few hundred milliseconds. Fesenko and Lyubarskiy suggest that the "mechanism probably involves either a conformational transition in rhodopsin after the formation of metarhodopsin II, or a re-arrangement of photoreceptor membrane occurring, following a certain delay, after the formation of metarohodopsin II." What is interesting here is that the time-course of the conductance change does not match with the spectrally measurable visual pigment changes. In addition when the high conductance state has decayed, a low, but enhanced, conductance state remains. This probably corresponds to the conductance identified by Montal et al. (1977). The study of invertebrate visual pigments using the methods of Montal et al. and

Fesenko and Lyubarskiy should be extremely interesting!

To summarise, a biophysical model of phototransduction and the afterpotential in R1-R6 of Drosophila has been formulated. The model arises from experimental observations and makes testable predictions. Curiously, the model departs considerably from available models of phototransduction in invertebrates (notably the "excitor-inhibitor" model) yet is very simple. Support for the model can be found in recent work on phototransduction in vertebrates, while much of the invertebrate work is clouded by the "excitor-inhibitor" model.

In general terms the vertebrate photoreceptors are similar to those of invertebrate species (some differences were described in chapter 1). Do the results in the thesis have any value in relation to these photoreceptors? Intracellular recordings from vertebrate photoreceptors confirm that their response is a sigmoid function of the logarithm of light intensity and can be described by equations similar to those given in chapter 2 (e.g. monkey cones: Boynton and Whitten, 1970; rods of axolotl: Grabowski et al., 1972). Both in the presence of ambient light (Boynton and Whitten, 1970) and after pigment bleaching (Grabowski et al., 1972) there are indications that "threshold" changes in parallel with changes in membrane potential. The analysis in chapters 2 and 3 suggest that it would be more useful to examine the relationship between response amplitude and membrane potential. The difficulty with either of these comparisons is the scarcity of accurate d.c. recordings. For instance Lauglin (1975) cited only three cases of "correctly" executed intracellular studies of invertebrates. Surprisingly, here lies the advantage of the ERG of Drosophila because stable d.c. recordings can be obtained relatively

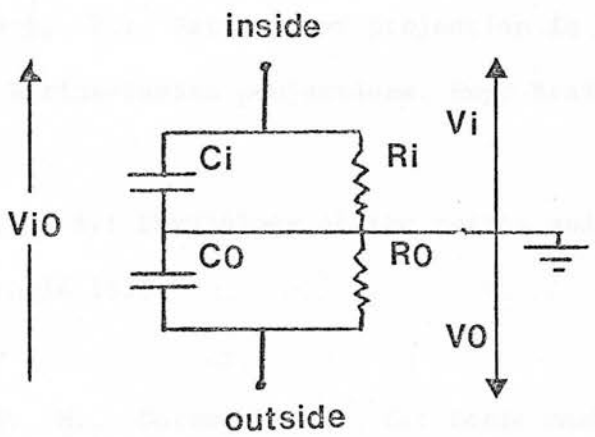
easily.

To explain the d.c. stability of the ERG of Drosophila, and the fact that intracellular and extracellular records are so similar (there are no transmembrane recordings from Drosophila), chapter 6 examined a model of extracellular potentials which does not rely on the concept of "current flow through an extracellular resistor". The ERG of Drosophila can be stable for several hours. Chapter 6 demonstrated that extracellular potentials can exist without extracellular current flow when the cells lie in an ideal electrical compartment. How closely the compartments of the Drosophila visual system (Heisenberg, 1971) correspond to ideal compartments remains to be seen. Shaw (1975), for instance, has presented an alternative model, similar in concept to the capacitive model of chapter 6, but relying on resistances to "virtual earth". Shaw's model must apply as an equilibrium at infinite time because no parts of an animal body can be separated by infinite resistance. On the other hand, Shaw's own results for the locust eye suggest that the exchange of charge between "compartments" is very small (Shaw, 1977). The capacitive model must apply at $t = 0$, when the exchange of charge between compartments is infinitely small. The question which then arises is: how much time is required for the potentials of spaces within a less than ideal compartment to come to the resistive equilibrium? If it is very large compared to the rise time of the transmembrane potential, then the capacitive equilibrium will be most evident. If it is small compared to the rise time of the transmembrane potential, then the capacitive equilibrium will be obscured and the resistive equilibrium will predominate. If it is a few seconds, the capacitive equilibrium will predominate initially and the extracellular and intracellular potentials will sweep towards the resistive equilibrium. Presumably,

each eye must have a characteristic time constant which determines whether the capacitive equilibrium is conspicuous.

The intracellular and extracellular recordings from Sepiola (Pynsent and Duncan, 1977) are extremely interesting in this context. They show a transient peak in the intracellular records which is complemented by a trough in the extracellular recordings. The reconstructed transmembrane potential is approximately a square wave (Pynsent and Duncan, 1977 Fig. 2 row 1). This transient may arise because at t = 0 the capacitive model applies, while within a few seconds the resistive equilibrium is reached and the resistive model applies. Certainly, potentials similar to those shown by Pynsent and Duncan can be obtained by applying a square wave to the terminals of a network such as shown in Figure 1. This network combines the capacitive model of chapter 6 with the resistive model of Shaw (1975).

Figure 1: A combination of the capacitive model of chapter 6 and the resistive model of Shaw (1975) to account for extracellular potentials. The equilibrium solutions for the ratio of the extracelluar and intracellular potentials after a disturbance of the transmembrane potential are different unless Ci/C0 = R0/Ri. The capacitive equilibrium, which will apply at very small time intervals after the disturbance so that the conditions of an ideal compartment are satisfied, is given by V0/Vi = -Ci/C0. The resistive equilibrium, which will apply at larger time intervals is given by V0/Vi = -R0/Ri.



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Journal of Comparative Physiology · A

Blue-Adaptation and Orange-Adaptation in White-eyed *Drosophila*: Evidence that the Prolonged Afterpotential is Correlated with the Amount of M580 in R_{1-6}

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Summary. 1. Short-wavelength light produces prolonged changes in the ERG afterpotential and the size of the ERG response to a fixed testflash; these changes may be reversed at any time by long-wavelength light: this 'blue/orange adaptation' is clearly separable from other components of light or dark adaptation (Fig. 3), and operates over a totally different time scale (Fig. 10a, b).

2. The amount of light required to produce a criterion change in the state of blue/orange adaptation of the eye is independent of the intensity or temporal pattern of the stimulus (Figs. 1, 2, 3, 4, 5, 6 and Table 2).

3. Recovery of sensitivity in darkness following an intense blue stimulus is extremely slow (hours); and at any time during the recovery of sensitivity, blue light will again diminish sensitivity or orange light restore it (Fig. 10a, b).

4. The response amplitude elicited by a testflash is highly negatively correlated with the size of the ERG afterpotential (a) during blue-adaptation (Figs. 1, 2, 3), (b) during the decline of the ERG afterpotential resulting from exposure of the blue-adapted eye to long-wavelength light (Figs. 4, 5, 6, 7), (c) during dark-recovery from blue-adaptation.

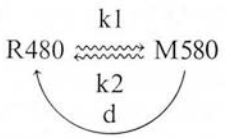
5. The data presented are consistent with the view that the adaptational changes described result from interconversions of R480 and M580; that the size of the prolonged ERG afterpotential is closely linked to the amount of M580 in R_{1-6} following transduction; that the effects observed in the ERG closely reflect events occurring across the transducing membranes of R_{1-6}; and that there is a component of the membrane conductance of R_{1-6} that is correlated with the presence of M580.

Introduction

The ommatidia of the dipteran compound eye contain an unfused rhabdom: the rhabdomeres of individual retinula cells are separate along their entire length

and in addition are arranged in a typical asymmetric pattern. Pak (1975) has recently reviewed the relevant anatomy of the fly retina with particular reference to *Drosophila*. Kirschfeld and Franceschini examined the optics of the compound eye of *Musca* and concluded that it contains two anatomically and functionally distinct systems: one system receives input from the central retinula cells (R_{7+8}), and the second receives input from the peripheral retinula cells (R_{1-6}) (reviewed in Kirschfeld, 1969). This division into two systems appears to be a general property of the dipteran compound eye (McCann and Arnett, 1972) and the two systems differ in their spectral sensitivity: R_{1-6} are maximally sensitive to green light (470 nm to 510 nm) with a second peak in the ultra-violet (340 nm to 360 nm), while R_{7+8} are maximally sensitive to blue light (around 460 nm) and also to UV (*Musca*, *Calliphora* and *Phaenicia*: McCann and Arnett, 1972; *Drosophila*: Minke et al., 1975 and Harris et al., 1976). Using morphological mutants of *Drosophila* Harris et al. (1976) demonstrated that R_7 is exclusively a UV receptor; a single peaked UV receptor (either R_7 or R_8) has also been reported for *Calliphora* (Meffert and Smola, 1976).

The major visual pigments of the *Drosophila* eye were identified by Ostroy et al. (1974) as an R480 and an M580 which is stable in darkness (time-constant about 6 h: Pak and Lidington, 1974) suggesting the simple pigment system



where the wavy lines represent photochemical reactions with rate-constants k1 and k2, which are intensity-dependent, and the solid line represents the dark-regeneration of R480 with rate-constant d. Phenomena associated with this pigment system (Pak and Lidington, 1974) have been localised to R_{1-6} using intracellular recording (Minke et al., 1975). A prolonged depolarising afterpotential (PDA) is accompanied by a reduction in the sensitivity of R_{1-6} following an intense blue stimulus; the PDA is abolished by orange light. The ERG of white-eyed *Drosophila* reveals similar phenomena: an intense blue stimulus is followed by a prolonged corneal negative potential (here called a *prolonged corneal negative afterpotential*, PCNA) which rapidly declines during or after an intense orange stimulus; during the PCNA a residual monophasic response is observed in the ERG (Cosens and Briscoe, 1972). The intracellular evidence of PDA in R_{1-6} (Minke et al., 1975), and ERG data from mutants lacking R_{1-6} (Harris et al., 1976) confirm the interpretation of the PCNA presented by Cosens and Wright (1975) and Minke et al. (1975): during a PCNA the contribution of R_{1-6} to the ERG is reduced, exposing the monophasic response of the central retinula cells (the superimposed response, SR of Minke et al., 1975). Harris' study shows that R_{1-6} contain the pigment system drawn above; R_7 contains a similar system with an R370 and an M470; while R_8 is "a non-adapting blue-receptor with a third type of rhodopsin" (Harris et al., 1976).

This paper investigates the effects of short-wavelength light (termed *blue-adaptation*) and long-wavelength light (termed *orange-adaptation*) upon R_{1-6} by observing the ERG of white-eyed *Drosophila*.

An apparently similar type of antagonistic adaptation associated with interconversions of dark-stable visual pigments has been investigated in the lateral ocelli of *Balanus* (Hochstein et al., 1973) and the median eye of *Limulus* (Nolte and Brown, 1972; Minke et al., 1973). In these preparations it has been demonstrated that a stimulus which generates a substantial amount of M is followed by a PDA. This PDA declines in darkness in a few seconds to thirty minutes, *and does so independently of dark-regeneration of R.* For some time after the PDA has declined the visual pigment is largely M, consequently a second M-favouring stimulus does not substantially change the amount of M present, and does not result in a PDA. An R-regenerating stimulus will reduce M and abolish any remaining PDA, or, if applied after the PDA has declined, will, by regenerating R, permit a fresh PDA following an M-generating stimulus. Two distinct adaptational states thus exist dependent upon the visual pigment composition. A PDA marks the conversion between the low and high metarhodopsin states, but its duration does not equal that of the underlying visual pigment changes. This separation of the PDA from the statics of the visual pigment composition is part of the evidence used to construct a model of transduction involving *excitatory and inhibitory transmitters* released by photopigment transitions. According to the model the transmitters interact to produce depolarisation of the photoreceptors and they decay independently of (and more rapidly than) dark changes in the visual pigment composition (Hochstein et al., 1973; Minke et al., 1973).

The *Drosophila* visual system has not yet been examined in a way that is directly comparable with the studies of *Balanus* and *Limulus*. In particular the duration of the PCNA or the PDA has not been compared with the long time-constant for the recovery of sensitivity following blue adaptation in this species (ERG data: recovery of sensitivity takes more than 3 h: Cosens and Briscoe, 1972[1]). This time-course seems similar to the reported time-constant for dark-decay of M580 (greater than 6 h, data from the M-potential: Pak and Lidington, 1974) which suggests a possible correlation between sensitivity and R480; however Stavenga (1975) has questioned the long time-constant reported by Pak and Lidington. Hamdorf's group has argued that rhodopsin concentration controls the sensitivity of invertebrate visual receptor cells (reviewed in Hamdorf and Schwemer, 1975). However the importance of metarhodopsin concentration to the sensitivity of R_{1-6} in *Calliphora* has recently been demonstrated by Razmjoo and Hamdorf (1976). Our data show that (a) the decline of the PCNA in white-eyed *Drosophila* is extremely slow and accompanies dark changes in visual pigment composition (confirming Pak and Lidington's data on M580 decay) thus (b) the size of the afterpotential is correlated with the *amount* of M580 in R_{1-6}. We also show that (c) the size of the *ERG afterpotential* and response amplitude are highly negatively correlated in the manner predicted for transmembrane potentials using a model of the transducing membrane. This and other evidence indicates that (d) both ERG and intracellular data concerned with blue/orange adaptation reflect transmembrane events. Points a, b and d suggest that (e) the *Drosophila* data is compatible with an 'excitor-inhibitor' model of transduction only if the lifetime of the 'excitor' is greater

[1] ERG and intracellular evidence: PDA/PCNA last several hours: Minke et al., 1975

than the lifetime of M580 (that is the time-constant of dark-decay is several hours). We suggest the possibility that (f) the amount of M580 remaining after transduction determines (via a long-lived excitor?) the magnitude of the transmembrane afterpotential which in turn alters the size of the measured response amplitude to a fixed stimulus (and hence sensitivity) *independently of sensitivity changes attributable to the concentration of R480*. That is, sensitivity is negatively correlated with the concentration of M580.

Materials and Methods

1. The Flies Used, and Recording Technique

The materials and ERG recording technique used were similar to those previously reported from this laboratory (Cosens, 1971; Cosens and Wright, 1975). The flies were white-eyed mutants (w or bw/cn) of *Drosophila melanogaster* which, in contrast to the red-eyed wild-type, may be readily blue/orange adapted (Cosens and Briscoe, 1972). They were cultured at room temperature (ca. 22 °C) on standard cornmeal-agar-molasses medium. ERGs from intact flies were monitored on a Telequipment D53 oscilloscope and a Bryans 28000 penwriter via an extracellular Ringer, or sodium chloride, filled glass micropipette. The micropipette was inserted through the cornea into the retinula cell region of the eye and led off, via an Ag/AgCl electrode, to a high impedance buffer amplifier. A gold wire inserted into the thorax served as the indifferent electrode.

2. Light Sources

The preparations were bathed with light using a collimated beam from 100 W 12 V tungsten-halogen source (Philips Al/215) which we call the *adapting light*. In addition a dimmer light, the *testflash*, was focussed onto the eye from an overdriven 2.5 V, 0.3 A, torch bulb. This was used for presenting repetitive stimuli and was controlled by a multivibrator with two fixed periods: 2 s and 4 s. The duration of the 'on-time' was variable from a minimum of 180 ms.

With both light sources the wavelength of irradiation was controlled by Balzers broad-band spectral filters. Table 1 shows the transmission characteristics (bandwidth to 10% transmission) of these filters measured with a Pye Unicam SP800 spectrophotometer. The maximum energy emitted at each waveband was measured for both light sources using a Tektronics J16 Digital Photometer and J6502 probe calibrated in $mW\,m^{-2}$. Measurement of this parameter is subject to some error particularly with the testflash, the focussed beam of which does not fill the field of view of the probe. Correction for this is included in the measurements listed in the table. Photon flux was calculated from the relationship: $E=hc/\lambda$, using the midband wavelength of the appropriate filter.

Table 1. Characteristics of the filters and light sources used in the experiments

Filter colour	10% bandwidth (nm)	Adapting light intensity 10^{16} photons $cm^{-2}s^{-1}$	Testflash intensity 10^{15} photons $cm^{-2}s^{-1}$
violet	316–425	0.2	0.8
blue	416–500	1.4	0.8
green	466–546	1.6	1.4
yellow	520–600	3.6	6.3
orange	563–644	2.2	6.2
red	606–670	2.3	6.5
deep red	670–740	1.8	5.9

The intensity of illumination was controlled by calibrated heat-fogged photographic plates or Balzers neutral density filters and a Balzers Calflex B1K1 interference heat filter was used at all times.

3. Experimental Procedure

Three illumination regimes were used in the experiments reported: A) Using the adapting light controlled by a photographic leaf-shutter: Type 1. Prolonged continuous illumination. Type 2. Brief pulses of illumination separated, or followed, by periods of darkness. B) Using the testflash: Type 3. Repetitive alternation of illumination and darkness.

It is a standard aim when testing the response of a receptor to avoid the possibility that the test-stimulus changes its state of adaptation, this involves the use of brief dim stimuli. In a number of the experiments reported we have deliberately used bright test-stimuli which do adapt the receptor. This has allowed us to monitor the receptor response continuously during the resultant adaptation. When we have used the testflash in this way we have called it the *adapting-flash* (see Figs. 3, 5, 6, 7). Using an adapting-flash together with a continuous adapting light we have observed receptor response when stimulated by two adapting wavelengths (Figs. 8, 9).

The eyes were selectively adapted using the colour regimes described in Cosens and Wright (1975). These authors, Minke et al. (1975) and Harris et al. (1976) have demonstrated that the phenomena observed (see Fig. 1) are attributable to retinula cells 1 to 6. Thus by observing changes in the ERG *during* blue and orange adaptation we are principally examining the behaviour of this cell group.

We have made two basic measurements from our ERG records: the first is the conventional measure of the *response amplitude* elicited by a fixed intensity stimulus and is an indicator of *sensitivity*; the second is a measurement of the ERG baseline relative to an arbitrary potential level in the eye, we have used the term *afterpotential* to describe this measurement. We have adopted the convention of displaying corneal negative potentials upwards on our figures and after-potentials are measured as deviations of the ERG baseline in a corneal negative direction from an arbitrary potential in the region of the initial (pre-blue-adaptation) level of this baseline. Exactly how these measurements were made is clarified in Figure 1 and the accompanying text.

Results

Blue-Adaptation and Orange-Adaptation in the White-eyed Mutant of Drosophila

This section describes the effects of an intense blue stimulus upon the ERG of a 'dark-adapted' *Drosophila* eye, and the reversal of these effects following an orange stimulus.

The time-constant (T: time required for a 50% change) for the decline of ERG baseline (*afterpotential*) following any dose of a long-wavelength stimulus, or a small dose of a short-wavelength stimulus, rarely exceeds 3 s in a fresh preparation. The recovery of sensitivity can be somewhat slower particularly following an intense stimulus: T = 10 to 30 s. These time-constants can be vastly enlarged following a large dose of short-wavelength light. We will call this effect, and any other effects specific to short-wavelength light in the blue region (i.e. excluding UV): blue-adaptation. Figure 1, a penwriter record, illustrates some of the properties of blue-adaptation. A brief, dim, white light was flashing throughout this demonstration experiment to indicate changes in the response of the eye to a constant stimulus (an indicator of sensitivity). Following an intense blue stimulus delivered at *A* a *prolonged corneal negative afterpotential*

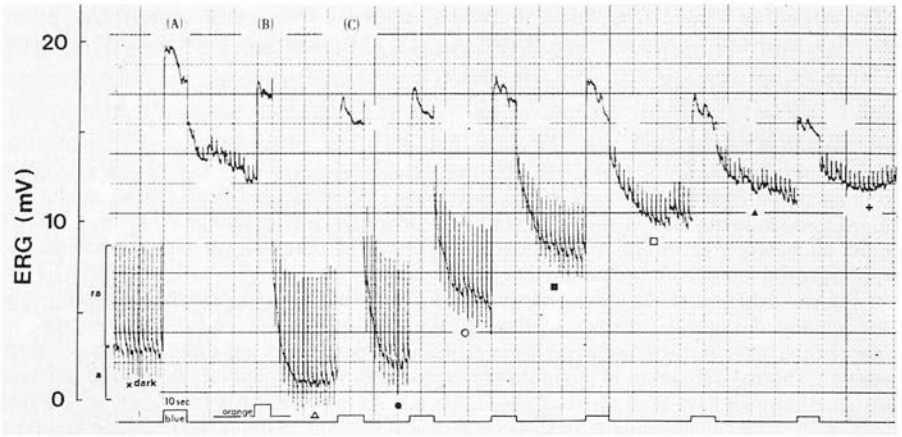


Fig. 1. A penwriter record of the ERG of a white-eyed *Drosophila* demonstrating *A* blue-adaptation, *B* the reversal of blue-adaptation with orange light, and *C* that blue-adaptation is cumulative when a reduced intensity of blue light is used; the experiment is described fully in the text. At the left hand side of the figure we show how we measure the *ERG baseline (afterpotential: a)* from an arbitrary potential level, and the *response amplitude (ra)* elicited by a testflash

(PCNA) is seen, accompanied by a considerable reduction in the size of the response to the testflash. This response is now *monophasic* (the *superimposed response*, SR of Minke et al., 1975) and lacks the lamina on-transient which was previously visible. A point to note is that we have adopted the convention of plotting our ERG data corneal upwards, this facilitates the comparison of extracellular and intracellular recordings.

The slow decline of afterpotential in the blue-adapted eye may be contrasted with the rapid decline of the afterpotential, and the recovery of the response to the testflash, following intense orange light delivered at *B* on the record. For the remainder of this paper we will call the adaptation specific to long-wavelength light in the orange region: *orange-adaptation.*

The references cited in the introduction show that during the PCNA in a blue-adapted eye R_{1-6} are depolarised and unresponsive while R_{7+8} are unaffected. Since our 'white' testflash contains little or no UV light (to which R_7 is exclusively responsive: Harris et al., 1976) we conclude that the SR visible during the PCNA is the response of R_8.

In view of the type of data reported in this section (which has been previously described in various ways in a number of reports of the electrophysiology of white-eyed *Drosophila*: Cosens and Briscoe, 1972; Cosens and Wright, 1975; Minke et al., 1975; Stark, 1975; and Harris et al., 1976) it is clearly important at all times to be aware of the state of spectral adaptation of the eye. The remainder of the results are concerned with the parameters and properties of blue and orange adaptation; prior to each of the experiments the eyes were dosed with large amounts of either long-wavelength or short-wavelength light (pre-adaptation) which we consider sufficient to specify the eye as either orange or blue adapted. The next section examines blue-adaptation with particular

reference to the effect of the *amount* of blue light delivered in the stimulus (dose); prior to all of the experiments in this section the eye was orange-adapted with a large dose of long-wavelength light.

Blue-Adaptation

Part *C* of Figure 1 shows the ERG response when successive doses of blue light of reduced intensity are applied to the eye. The effect of the blue irradiation is cumulative upon (a) the time-constant of decline of the afterpotential, and hence upon (b) the size of the afterpotential measured after an arbitrary fixed time, and also upon (c) the size of the response to the testflash. The afterpotential and the response amplitude are highly negatively correlated; Figure 2 is a graph of measurements from Figure 1 which illustrates this relationship ($r=0.98$, $df=63$, $p<0.01$ for the data from part *C* of Fig. 1). The source of the points on the graph is indicated by the symbols, which are common to Figures 1 and 2. The symbol 'X' shows the mean value of measurements of afterpotential and response amplitude at the start of the experiment (eye 'dark-adapted'); the symbol '+' shows the mean of these measurements after the sixth dose of blue light (eye blue-adapted and showing a saturating PCNA).

A correlation between ERG afterpotential and response amplitude appears to be a consistent property of the ERG response and will be observed repeatedly in the remaining results.

The dynamics of blue-adaptation are clearly separable from those of spectrally non-specific light-adaptation: Figure 3, again penwriter records, illustrates this point. The bottom record (*row 4* of Fig. 3) shows, in sequence, the ERG response to (a) a 10 s intense red stimulus (which orange-adapts the eye) followed by 15 s of darkness, (b) a bright *white* adapting-flash (1 s 'on':1 s 'off') which continued for several minutes, and was followed by 30 s of darkness, (c) a

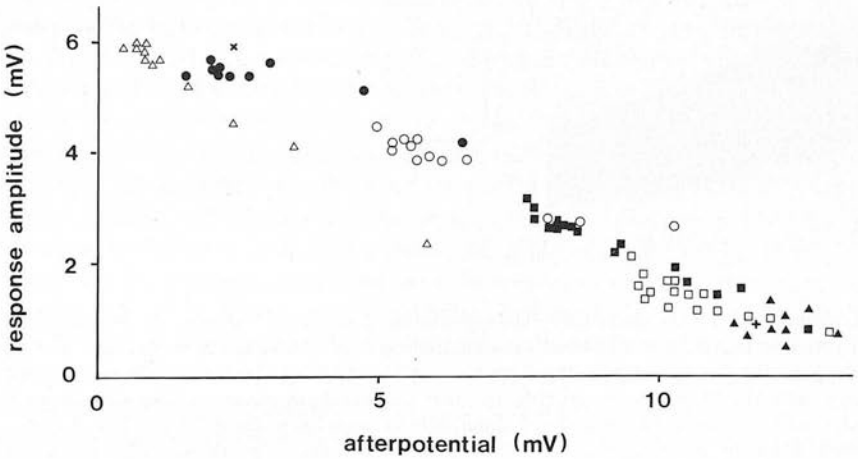


Fig. 2. A graph of the response amplitude to the testflash against the afterpotential during the experiments shown in Figure 1. The measurements were taken directly from Figure 1 and the symbols, which are common to Figures 1 and 2, indicate the source of the data

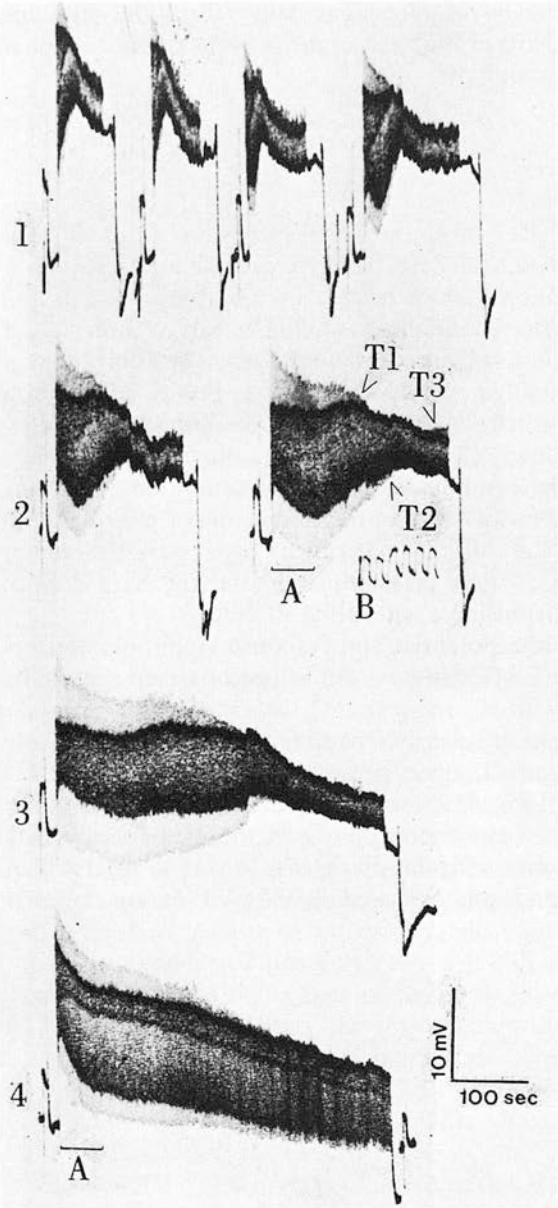


Fig. 3. A montage of ERGs elicited by flashing stimuli. Each record shows, in sequence, (a) the ERG response to an intense 10 s red stimulus, followed by 15 s of darkness (the eye is now orange-adapted), (b) the response to the flashing stimulus (1 s 'on': 1 s 'off'), followed by 30 s of darkness (note the PCNA in the records on *rows 1, 2 and 3*: responses to *blue* stimuli), and (c) the response to a second 10 s intense red stimulus (which abolishes the PCNAs). *Row 4* shows the response when the adapting-flash was *white* (note that there is no PCNA after the white adapting-flash). The records are interpreted fully in the text. *Rows 1, 2 and 3* show responses to *blue* adapting-flashes of seven intensities (*row 1*: 7.8, 6.2, 4.9, 1.9; *row 2*: 1.2, 0.78; *row 3*: 0.39, $\times 10^{14}$ photons $cm^{-2}s^{-1}$)

second 10 s intense red stimulus, followed by a brief period of darkness after which the record ends. (Note that in the record of *row 4* the penwriter DC offset was used to shift the baseline upwards just before this second red stimulus: this shift is of no biological significance. It was necessary in this preparation due to the steady downward drift visible in all of the records of Figure 3. (this drift is not typically observed.) The broad shaded band forming the major part of each of the records in Figure 3 is the response to the flashing stimulus. The darker central (horizontal) band is the sustained portion of the response attributable to the activity of the retinula cells (top border = the maximum of the corneal negative response due to R_{1-8}; the lower border = the residual afterpotential at the end of the 1 s dark period which separates the flashes) and the lighter fringes above and below this central band reflect the activity of the lamina (the lamina-origin transients are attenuated due to the limited slew-rate of the penwriter: upper fringe = lamina off-transient; lower fringe = lamina on-transient). Returning specifically to the response to a *white* flashing light (*row 4* on Fig. 3), considerable changes in response (light-adaptation) occur during the first minute or so of the stimulus: the lower border (afterpotential) after 1 s of darkness) rises and then falls accompanied by negatively correlated changes in the response amplitude (marked A on Fig. 3). As the stimulation proceeds the lamina transients can be seen to decline progressively (this effect is not truly light-adaptation: the lamina transients are restored after either periods of continuous darkness or illumination; the effect is thus a kind of 'fatigue' due to the fact that the light is flashing, Wright, in prep.). When the flashing stimulus is terminated the afterpotential declines rapidly; and a red stimulus 30 s later induces no further decline (i.e. there is no PCNA).

Contrast the light-adaptation described in the previous paragraph with the adaptation visible on the remaining records of Figure 3 (*rows 1 to 3*). The stimulus regime resulting in these records was similar to that described above except that the flashing stimulus was a *blue* light of a range of intensities (*row 1*: 7.8, 6.2, 4.9, 1.9; *row 2*: 1.2, 0.78; *row 3*: 0.39, $\times 10^{14}$ photons $cm^{-2}s^{-1}$). Again non-specific light-adaptation is visible, particularly on the second record of *row 2* (marked A) but a new component appears: blue-adaptation. Blue-adaptation is visible in the records of Figure 3 as (a) an asymptotic, or overshooting, rise of the afterpotential (lower border of the dark, central band of the ERG) accompanied by (b) a negatively correlated progressive decline of response amplitude and, in sequence, (c) the gradual elimination of the lamina off-transient (upper fringe) and (d) the lamina on-transient (lower fringe) followed by (e) the appearance of a new on-transient, of slower speed, *T3* on Figure 3. The afterpotential changes associated with blue-adaptation are prolonged, for instance a PCNA is visible when the blue flashing light is switched off (*rows 1 to 3* on Fig. 3) and decline during and after the second 10 s red stimulus. The monophasic nature of the SR visible on these records (see also inset B) and the saturating, or near saturating, PCNAs during the 30 s dark period following the blue-adaptation show that R_{1-6} are almost completely unresponsive after the doses we have applied. Additional data to be published at a later date shows that intermediate doses of blue light result in intermediate magnitudes of PCNA (Wright, in prep.).

Table 2. Inverse correlations between intensity and duration of blue light required to produce three criterion changes in ERG response

Criterion	Label on Figure 3	r	df	p
loss of lamina off-transient	*T1*	0.99	12	<0.01
loss of lamina on-transient	*T2*	0.99	12	<0.01
appearance of new on-transient	*T3*	0.98	10	<0.01

Stark (1975) reported that, over a wide range of intensities, the amount of blue light required to produce a criterion change in ERG response is independent of the intensity of light used. Our data are in agreement with his finding: we find a high correlation between the duration of the illumination required to produce three different criterion changes in the ERG and the reciprocal of the intensity of blue light inducing the adaptation. Correlations obtained using data from Figure 3 and a replicate with the same preparation are tabulated above.

Thus the critical parameter determining the extent of blue-adaptation is the *amount* of blue light in the stimulus. This implies that the progress of blue-adaptation is related to a simple photochemical conversion creating a product which has a large dark-decay time-constant: presumably the generation of M580 in R_{1-6} which behaves in this way (Harris et al., 1976); the time-constant reported for the dark-decay of M580 is about 6 h (Pak and Lidington, 1974). In our experimental situation about 10^{16} photons cm^{-2} are sufficient to produce a saturating PCNA.

Orange-Adaptation of Blue-adapted Eyes

In this section we report the behaviour of the ERG during orange-adaptation of blue-adapted eyes, when we presume that R480 is regenerated in R_{1-6} (Harris et al., 1976).

The three types of illumination regime described in the methods were used to orange-adapt blue-adapted eyes. The following key points emerge: (a) in each case the curve of afterpotential decline is *sigmoid*, (b) the curve of recovery of response amplitude is also sigmoid and follows a similar (mirror image) time course to afterpotential decline, (c) response amplitude to a fixed intensity stimulus is negatively correlated with the size of the afterpotential and (d) progress along the sigmoid curve of afterpotential decline is determined by the dose of orange light delivered in the adapting stimulus (time for 50% decline of afterpotential is inversely proportional to the intensity of illumination used for adaptation).

Figure 4 shows data from an experiment in which blue-adapted eyes were orange-adapted with continuous red light. The intensity of red light producing the changes visible in *curves 1, 2, 3, 4 and 5* was 23.2, 2.32, 1.07, 0.56, 0.23, $\times 10^{15}$ photons $cm^{-2}s^{-1}$, respectively. Figure 4a shows the response amplitude elicited by a constant intensity white testflash (measured as shown in inset c) and

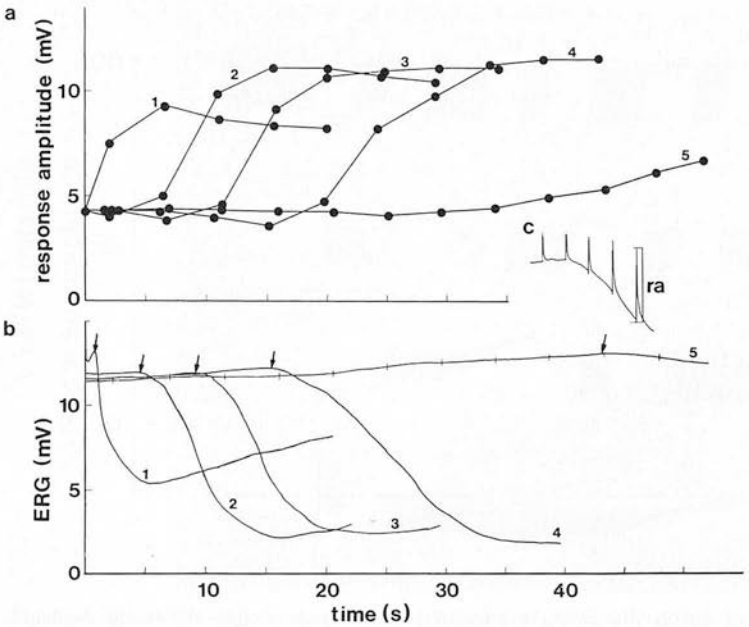


Fig. 4a and b. Changes in the ERG of the blue-adapted eye as it is orange-adapted by a continuous red light. **a** shows measurements of the response amplitude elicited by a white testflash; **b** is drawn from tracings of the ERG baseline: the ordinate is measured relative to the orange-adapted and dark-adapted level of the baseline; the curves thus show the afterpotential. The data plotted was obtained using 5 intensities of red light to orange-adapt the blue-adapted eyes; the red intensities resulting in the *curves 1, 2, 3, 4 and 5* were 23.2, 2.32, 1.07, 0.56, 0.23, $\times 10^{15}$ photons $cm^{-2}s^{-1}$. The arrows on Figure 4b indicate that an equal number of photons has been delivered to the preparations when the decline of afterpotential begins

Figure 4b was compiled from tracings of the ERG baseline, during the red illumination in each experiment. In each case the red illumination caused a decline in ERG baseline (afterpotential) along a sigmoid curve. The final level of the baseline is equivalent to the *stimulus-coincident* response from the orange-adapted eye, curve 5 reached this level in about 90 s. The duration of the adapting illumination required for 50% of the observed decline of the afterpotential is inversely correlated with its intensity ($r=0.99$, $df=3$, $p<0.01$) and, as can be seen from the figure, afterpotential and response amplitude follow a similar (mirror image) curve; and they are thus negatively correlated. The arrows on Figure 4b indicate the delivery of an equal number of photons for the respective curves: their location with respect to the beginning of the decline of afterpotential illustrates the importance of the dose of long-wavelength light in determining the extent of orange-adaptation.

Figure 5 is a montage of ERGs from a series of experiments using an orange adapting-flash. Prior to each record the eye was orange-adapted and dark-adapted. The illumination sequence which produced the ERGs shown was: (a) orange adapting-flash (1 s 'on': 1 s 'off') applied, then switched off, (b) 10 s of intense blue light applied and followed by 30 s of darkness (note the

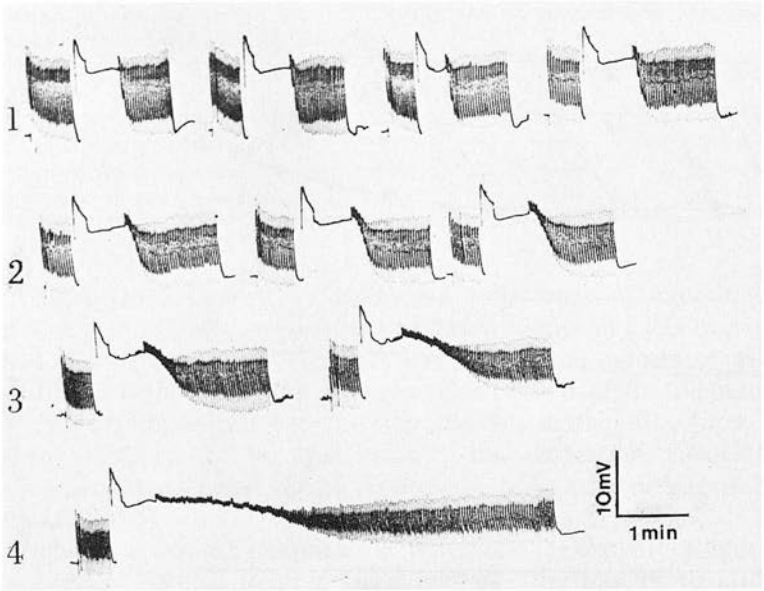


Fig. 5. A montage of ERGs illustrating orange-adaptation. Each record shows, in sequence, the response of the eye to (a) an orange adapting-flash (1 s 'on': 1 s 'off'), followed by a brief dark period, (b) an intense 10 s blue stimulus, followed by 30 s of darkness (note the PCNA in the now blue-adapted eye), and (c) the re-applied orange adapting-flash. The records were obtained using 10 intensities of orange adapting-flash (*row 1*: 6.2, 4.9, 3.9, 3.1; *row 2*: 1.49, 0.99, 0.6; *row 3*: 0.29, 0.15; *row 4*: 0.05, $\times 10^{15}$ photons $cm^{-2}s^{-1}$)

PCNA in the now blue-adapted eye), (c) orange adapting-flash re-applied – inducing the decline of afterpotential and the recovery of response amplitude – and finally switched off. The montage shows records obtained using a series of orange intensities: *row 1*: 6.2, 4.9, 3.9, 3.1; *row 2*: 1.49, 0.99, 0.6; *row 3*: 0.29, 0.15; *row 4:* 0.05, $\times 10^{15}$ photons $cm^{-2}s^{-1}$. The records illustrate again (a) the sigmoid decline of afterpotential and sigmoid recovery of response amplitude, (b) the dependence of the rate of these changes upon the intensity of illumination (time required for 50% of the observed decline in afterpotential is inversely correlated with the orange adapting-flash intensity: $r=0.994$, $df=8$, $p<0.01$), and (c) the negative correlation of afterpotential and response amplitude. This last correlation becomes increasingly *curvilinear* as the intensity of the flashes is reduced – a very dim flash is not seen to increase the response amplitude until the ERG afterpotential is substantially reduced (for example see the trace of *row 4* on Fig. 5). A possible interpretation of this is considered in the general Discussion. Despite this effect, high linear correlations between afterpotential and response amplitude are obtained from all of the data in Figure 5, with no correlation coefficient worse than $r=0.95$ ($df=5$ to 25, $p<0.01$).

Figure 6 and 7 illustrate the key points we wish to emphasize in this section. Both of the figures are drawn from the same data from experiments of the type shown in Figure 5. Data for two intensities of an orange adapting-flash (180 ms

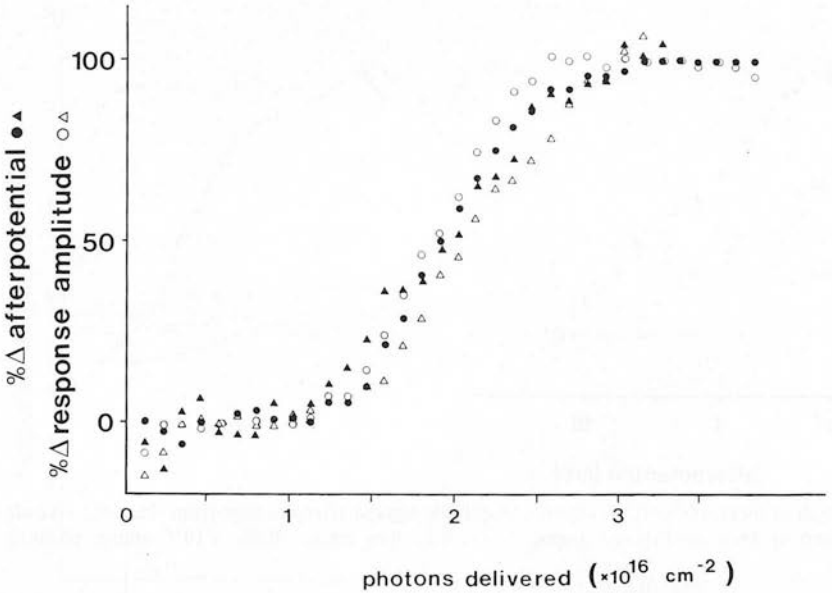


Fig. 6. A graph showing the percentage change in the ERG afterpotential (*solid symbols*), and the percentage change in the response amplitude to an orange adapting-flash (*open symbols*), during orange-adaptation against the number of orange photons delivered to a blue-adapted eye. The graph is derived from measurements of records of the type shown in Figure 5. Curves from experiments using two intensities of adapting-flash are defined by the symbols: *triangles*: 6.2; *circles* 0.62, $\times 10^{15}$ photons $cm^{-2}s^{-1}$

'on': 1,820 ms 'off') have been plotted: 6.2×10^{15} photons $cm^{-2}s^{-1}$ and one tenth of this. Figure 6 shows percentage change in afterpotential (*solid symbols*) and percentage change in response amplitude (*open symbols*), associated with orange adaptation, against photons delivered (i.e. the time scale of the curve for the lower intensity—*triangles*—is compressed by a factor of ten compared with the higher intensity—*circles*). Figure 7 is a graph of response amplitude against afterpotential during the same experiment (*solid circles* = higher intensity, *open circles* = lower intensity). Figure 6 clearly shows that the decline of the afterpotential is closely dependent upon the amount of orange light delivered to the eye (compare the curves defined by the *solid symbols*); and that the time-course of percentage change in response amplitude is very similar to that of percentage change in afterpotential (compare the *like symbols*—this relationship is increasingly distorted as testflash intensity is reduced). Figure 7 clearly shows the negative correlation between response amplitude and afterpotential and that the slope of the relationship is dependent upon the testflash intensity: this graph should be compared with the similar correlation observed during blue-adaption (Fig. 2).

The number of orange photons required to orange-adapt the eye is similar to the number of blue photons which result in full blue-adaptation (approximately 10^{16} photons cm^{-2}).

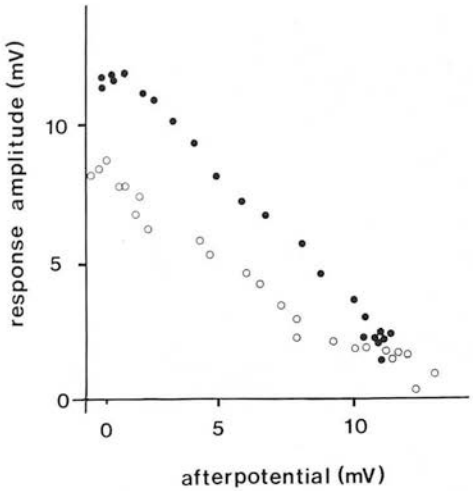


Fig. 7. A graph of measurements of response amplitude against afterpotential from the same records that were used to draw up Figure 6 (*solid circles*: 6.2; *open circles*: 0.62, $\times 10^{15}$ orange photons $cm^{-2}s^{-1}$)

The data so far presented show that the size of the afterpotential during blue and orange adaptation is sigmoidally related to the number of photons (either blue or orange) that have been absorbed by the visual pigment; and suggest that orange-adaptation simply reverses the process of blue-adaptation. It is implied that blue-adaptation is due to the accumulation of M580, that orange-adaptation is due to the elimination of M580 and consequent regeneration of R480, and that the PCNA is closely related to the visual pigment composition. The data further show that while a PCNA is present it is highly negatively correlated with (determines?) the size of the response to a testflash and hence the sensitivity of R_{1-6}; this relationship is further investigated in the next section and is termed the *afterpotential effect* (AE).

ERG Measurements during Exposure of the Eye to Two Antagonistic Wavelengths of Light

Classically the sensitivity of a photoreceptor is reduced during illumination with a background light, it should be clear from the preceeding results (e.g. Fig. 4) that this is not the case in *Drosophila* if a blue-adapted eye becomes orange-adapted by the background illumination. We saw (e.g. Fig. 3) that a short-wavelength adapting-flash results in a PCNA and a reduction in response size, to the SR (in this case the response of R_8); Figure 8 describes the changes in response amplitude when an orange-adapting background light is added to such an adapting-flash. The data begins with the eye blue-adapted at the moment when the orange-adapting background is added, both lights remained on throughout the experiment and the response amplitude plotted is the response

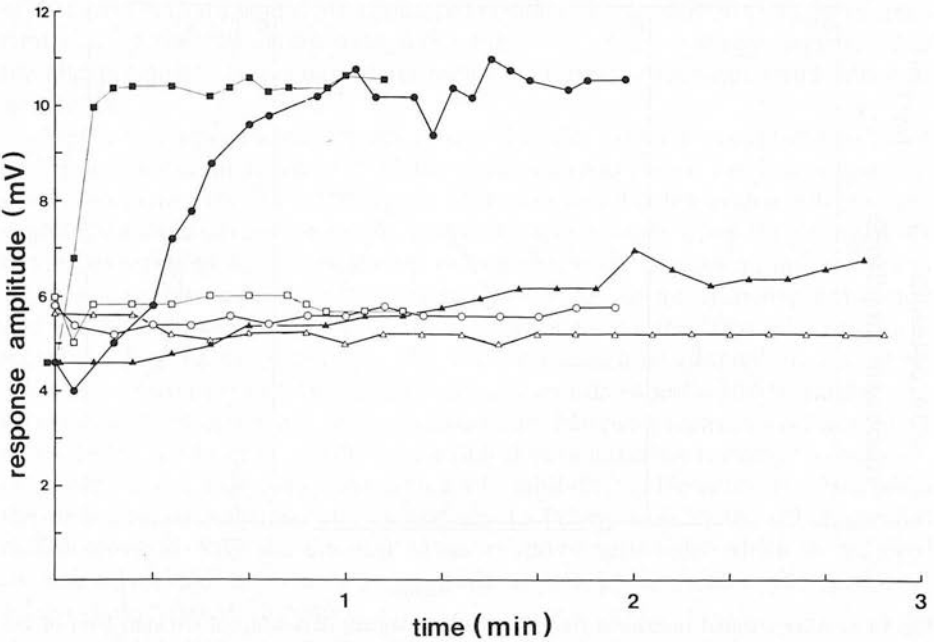


Fig. 8. A graph showing the time-course of the change in the response amplitude elicited by a green adapting-flash (which partially blue-adapts the eye) when a red background light is added. The *solid symbols* show measurements of the total response amplitude while the *open symbols* show measurements believed to correspond to the SR (the response of R_8) which was separately measurable in this particular preparation throughout the experiments. Data obtained using three intensities of red background are plotted in the figure (*squares*: 2.3; *circles*: 0.23; *triangles*: 0.023, $\times 10^{16}$ red photons cm $^{-2}s^{-1}$)

of the eye to the short-wavelength adapting-flash. The on-transient of the SR (the response of R_8) was identifiable throughout these experiments in this preparation, allowing continuous assessment of the contribution of R_8 to the ERG (*open symbols*). This response barely changes during the experiments and clearly R_8 is little affected. The total response (*solid symbols*) changes markedly and the response size *increases* during the long-wavelength illumination: presumably due to the elimination of M580 and regeneration of R480 in R_{1-6}. Curves for three intensities of red background light are shown (*squares*: 2.3; circles: 0.23; *triangles*: 0.023, $\times 10^{16}$ photons $cm^{-2}s^{-1}$) and it is clear from the figure that the equilibrium value of response amplitude and the rate at which this equilibrium is attained are dependent upon the intensity of the red illumination.

The results of Figure 8 could be interpreted as indicating that the response amplitude from, and hence the sensitivity of, R_{1-6} is determined entirely by the concentration of R480 following photochemical conversion by the red light. However, as before, we find that the afterpotential effect (AE) applies. Figure 9 demonstrates this by plotting data (mean $\pm$ 95% confidence limits, $n=10$) on the afterpotential (*squares*) and response amplitude (*circles*) from experiments similar to that described in Figure 8. The negative correlation of the AE is

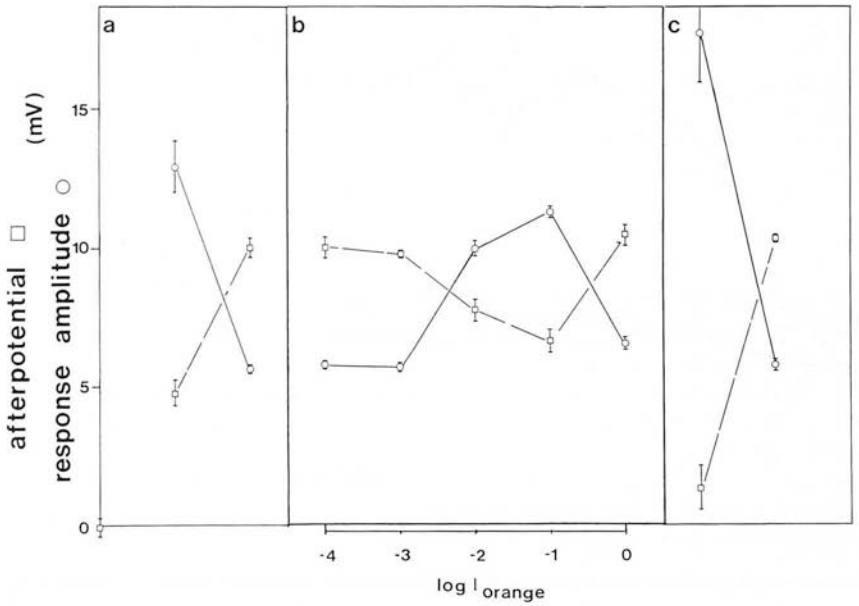


Fig. 9a–c. Afterpotential (measured from the orange-adapted, dark-adapted (10 min) level of the ERG baseline) and response amplitude elicited by a blue adapting-flash (8×10^{14} photons $cm^{-2}s^{-1}$) **a** of the first 10 responses to the blue adapting-flash and 10 responses when the eye became blue-adapted, **b** 1 min after an orange background light was added to the flashing blue light (the orange intensity at $logI_{orange}=0$ was 2.2×10^{16} photons $cm^{-2}s^{-1}$) and **c** identical to 'a' except for the prior adaptation, the data plotted here was obtained from the ERG of the eye immediately after the orange/blue mixing at the maximum orange intensity. Mean values ($\pm 95\%$ confidence limits, $n=10$) of measurements of the afterpotential (*squares*) and response amplitude (*circles*) are plotted on the figure

apparent from the Figure ($r=-0.97$, $df=7$, $p<0.01$). In these experiments a blue adapting-flash (8×10^{14} photons $cm^{-2}s^{-1}$) and an orange continuous background of 5 intensities ($log\ I_{orange}$ is relative to $I_{max}=2.2 \times 10^{16}$ photons $cm^{-2}s^{-1}$) were used (Fig. 9b), or the blue adapting-flash was used alone (Fig. 9a, c). Figure 9a shows measurements from an orange-adapted, and 10 min dark-adapted eye: at the origin the mean value of the dark-adapted afterpotential is shown; the response amplitude elicited by the first blue flash was 20.7 mV. Mean measurements of the first ten responses to the blue adapting flash, and of ten responses when the response had come to equilibrium (eye blue-adapted) are plotted. Measurements made 1 min after an orange background had been added to the blue adapting-flash illuminating a blue-adapted eye are plotted in Figure 9b. As the intensity of the orange light increases there is an increase in the response amplitude and a reduction in the size of the afterpotential. At the maximum intensity of the orange light the measured afterpotential rises due to the *stimulus-coincident* corneal negative response of the eye, and the response amplitude to the adapting-flash falls (this observation is typical of observations of response amplitude and ERG baseline [afterpotential] when, say, an orange background is added to an orange testflash); the measurements

of response amplitude and afterpotential at this intensity fall on the same regression line as the remaining data from Figure 9, which strongly suggests that the afterpotential is a primary determinant of response amplitude and hence of sensitivity.

Figure 9c shows measurements obtained under identical conditions to those of Figure 9a except in respect of the prior adaptation of the eye. Figure 9a shows data from the eye after 10 min of darkness following orange-adaptation: Figure 9c shows data obtained from the eye immediately after the orange/blue mixing experiment at the maximum orange intensity; the eye shows a marked *resistance* to blue-adaptation, as measured by the mean response amplitude and mean afterpotential of the first ten responses to the blue adapting-flash (compare Fig. 9a, c); eventually the eye becomes blue-adapted in the usual way. This resistance to blue-adaptation corresponds superficially to similar observations of the behaviour of the *Balanus* and *Limulus* preparations (Hochstein et al., 1973; Minke et al., 1973) for we find that in darkness it decays asymptotically with time: suggesting the action of 'inhibitors'. However in *Drosophila* the resistance is not absolute, as appears to be the case in the other species, and determines only the amount of short-wavelength light which is required to blue-adapt the eye. (A fuller account of this phenomenon will appear at a later date: Wright, in prep.)

Dark Changes in the ERG of Fully Blue-adapted Eyes

The independence of the decline of the PDA from the dark-decay of metarhodopsin, observed in the *Balanus* and *Limulus* preparations previously described (Hochstein et al., 1973; Minke et al., 1973), provided crucial evidence for the 'excitor-inhibitor' model of visual excitation in these species. The decline of the PCNA in a fully blue-adapted *Drosophila* eye is so protracted that it is difficult to make a continuous recording of it before (a) the preparation dies (after several hours) or (b) apparently spurious drifting of potential (either in the fly or in the recording equipment) is greater than the effect we are trying to observe. We therefore followed dark-recovery from blue-adaptation by monitoring the response of the eye to three dim green testflashes at various intervals after blue-adaptation. This method immediately detects problem 'a' as it arises, and largely overcomes problem 'b'; the afterpotential size can then be assessed at the points where we subsequently orange-adapt and/or blue-adapt the eye.

Figure 10 reports data obtained using experiments of the type described above. Figure 10a shows the time-course of the recovery of response amplitude elicited by the testflash (as a percentage of the initial [pre-blue] dark-adapted response size) after blue-adaptation (*circles*) and orange-adaptation (*squares*). These measurements were taken from the records of the experiment which is also the source of the data represented by the solid squares on Figure 10b which shows long-term changes in response amplitude (plotted as $(ra - ra_b)/(ra_o - ra_b) \times 100$ where ra = measured response amplitude; ra_b = ra 1 min after blue-adaptation; and ra_o = the [pre-blue] dark-adapted ra: thus the changes plot-

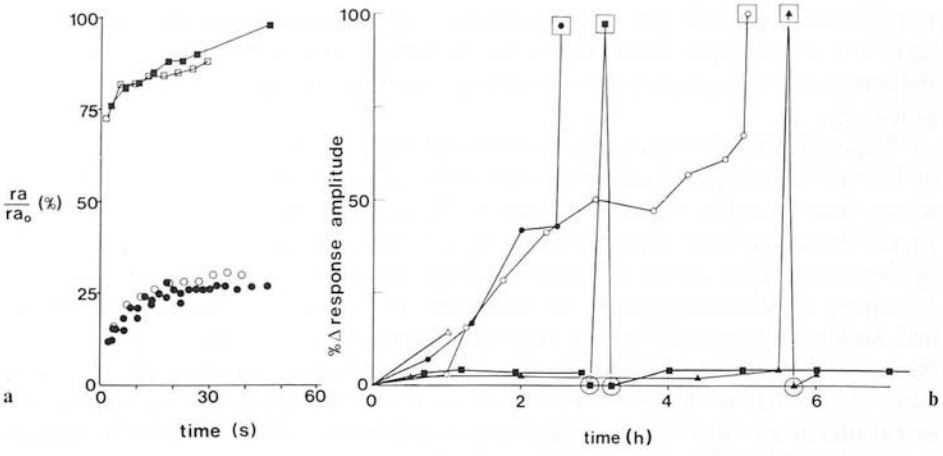


Fig. 10. a Short term changes in response amplitude (as a percentage of the dark-adapted [pre-blue] response amplitude, ra_o) after blue and orange illumination (long term changes from the same experiment are represented by squares in Fig. 10b). The *squares* show response amplitudes measured after the orange illumination (oversize square) on Figure 10b; the *circles* show measurements made after blue illumination (oversize circles) on Figure 10b. Data represented by *solid symbols* corresponds to the events shown on Figure 10b; the *open symbols* show data from events 10 h from the beginning of this experiment: the similarity of time-courses after long periods in the blue-adapted state is striking. **b** Percentage change in response amplitude occurring over long periods of darkness following blue-adaptation. The *oversize squares* show measurements made 1 min after an intense long-wavelength stimulus; *oversize circles* indicate measurements made 1 min after an intense short-wavelength stimulus. In calculating the data plotted on this figure the response amplitude measured 1 min after the initial blue-adaptation was subtracted; the recovery of response plotted on this figure is believed, therefore, to represent the recovery from blue-adaptation in R_{1-6}

ted are principally the reversal of blue-adaptation in R_{1-6}) after blue-adaptation. It is clear from Figure 10a that after blue-adaptation (*circles*) the recovery of the SR is near its asymptote within 1 min (ra_b) and that the shape of the recovery curve is similar after each of the blue-adapting stimuli (indicated by *oversize circles* on Fig. 10b); similarly the recovery of the orange-adapted response (*squares*) following long-wavelength illumination (indicated by *oversize squares* on Fig. 10b) is unchanged. The *open symbols* on Figure 10a plot data obtained ten hours after the beginning of the experiment.

Figure 10b shows the percentage of recovery of response amplitude in darkness following blue-adaptation, excluding the recovery occurring in the first minute: see Figure 10a. Data from 5 experiments of different duration are plotted on the graph; in each case recovery is very slow. At present we are not able to state whether the differences between the curves are due to (a) experimental differences (the faster recovering curves—*circles* and *open triangles*—might have been due to stray light reaching the preparation, causing photic regeneration of R480), (b) 'real' differences in recovery rates between flies, or (c) differences in the amount of M580 accumulated during the blue-adaptation; if this last is the case, and less M580 was present in the flies producing the faster recovering curves, then it is clear that the recovery of response ampli-

tude follows a sigmoid time-course. The following points emerge unambiguously from Figure 10b, (a) the recovery of response amplitude to a fixed stimulus is extremely slow (hours) and this time-course is of the same magnitude as that reported for the dark-decay of M580 (6 h: Pak and Lidington, 1974), (b) long-wavelength light restores response size at any stage of dark recovery (indicated by *oversize squares* on Fig. 10b; see also Fig. 10a), (c) short-wavelength light reduces response size to the blue-adapted level, after long periods of darkness following blue-adaptation or following orange adaptation (responses following short-wavelength adapting stimuli are marked by *oversize circles*).

These data indicate a close relationship between the state of blue/orange adaptation of the eye (measured by response to a testflash) and the visual pigment composition. We assessed the size of the afterpotential at the times when we switched the eye out of the blue-adapted state (*oversize squares* on Fig. 10b); although the overall size of the ERG response was sometimes reduced after long periods of blue-adaptation, a clear reduction in the size of the afterpotential was observed after the long-wavelength adaptation and the response amplitude and the size of the afterpotential were, again, highly negatively correlated: we conclude that the PCNA has a duration of several hours and that its magnitude can be predicted from the response amplitude data in Figure 10b.

This finding in white-eyed *Drosophila* contrasts sharply with the behaviour of the PDA reported in the *Balanus* and *Limulus* preparations (Hochstein et al., 1973; Minke et al., 1973). Our data confirm that the decay of M580 (Pak and Lidington, 1974) and the recovery of ERG sensitivity after blue-adaptation (Cosens and Briscoe, 1972) are processes that require several hours, and demonstrate that the decline of the ERG afterpotential parallels these processes. Minke et al. also reported that the PCNA and the PDA observed in *Drosophila* may last several hours (Minke et al., 1975). Thus in *Drosophila*, where both intracellular (PDA: Minke et al., 1975) and extracellular (PCNA: this paper and Minke et al., 1975) evidence of transmembrane afterpotentials in R_{1-6} exists, it is clear that measurements of sensitivity may be biased by the afterpotential effect, even several hours after spectral adaptation.

Discussion

It is apparent from the preceeding data that the blue/orange adapting behaviour of R_{1-6} in white-eyed *Drosophila* (judged from our ERG evidence) differs from the spectral adaptation behaviour of *Balanus* lateral ocelli and *Limulus* median eye (intracellular data: Hochstein et al., 1973; Minke et al., 1973). There are also some differences between our *Drosophila* data and the reported behaviour of R_{1-6} (intracellular recordings) in *Calliphora,* the other fly in which PDAs have been reported (Muijser et al., 1975). As in the *Balanus* and *Limulus* preparations, certain specific histories of spectral adaptation appear to be prerequisites if a PDA is to be observed: the specific nature of these histories argues in favour of the 'excitor-inhibitor' model of visual excitation applying in *Calliphora* (Muijser et al., 1975). We have found no evidence, in *Drosophila*, of states of the eye from which blue-adaptation will not arise given a sufficient

blue stimulus: PCNAs follow a blue stimulus (a) shortly after orange recovery from blue-adaptation (Figs. 1, 9c, 10a, b), (b) shortly after an orange pulse delivered to an orange-adapted eye (Figs. 3, 5), (c) after long periods of darkness following blue-adaptation (Fig. 10a, b), (d) after partial recovery from partial blue-adaptation (i.e. blue-adaptation is cumulative: Figs. 1, 2, 3) and (e) 10 min after orange-adaptation (Fig. 9a). We do observe quantitative differences in the *amount* of blue light required to produce a criterion change in the ERG (Wright, in prep.; and compare Fig. 9a, c). Except in 'old' preparations (where *all* aspects of the behaviour of the ERG are slowed down) we have found no states of the eye which orange-recovery from blue-adaptation is not possible. This evidence neither refutes nor corroborates the hypothesis that an 'excitor-inhibiting' interaction underlies visual excitation in R_{1-6} of *Drosophila*.

The Electrophysiological Behaviour of R_{1-6} in White-eyed Drosophila

A schematic description of the basic features of the electrophysiological behaviour of R_{1-6} is given in Figure 11. The description is based on the wavelength-specific effects on the rate of dark-decline of the afterpotential following a stimulus. The ERG fast declining response is assayed by sensitivity measurements and is maximally sensitive in the green region of the spectrum (λmax $\approx$ 490 nm: Cosens and Wright, 1975; Minke et al., 1975). The ERG slow-declining response (PCNA and PDA, i.e. the response associated with blue-adaptation) is most sensitive to blue light (λmax $\approx$ 460 nm) and is most readily reversed by orange light (λmax $\approx$ 580 nm: see Figure 5 in Stark, 1975). A much greater number of photons is required for blue-adaptation or orange-adaptation than are required to saturate the receptors with green light (Fig. 3).

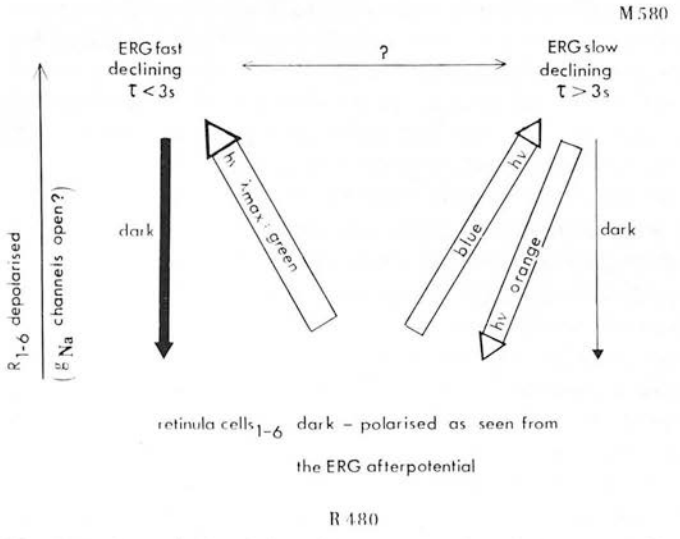


Fig. 11. A schematic description of the electrophysiological response of R_{1-6} in white-eyed *Drosophila*. See text for discussion

There is a striking similarity between the data associated with blue/orange adaptation which was obtained using extracellular electrodes (Fig. 1 in Cosens and Wright, 1975; Fig. 1 in Minke et al., 1975) and that obtained using intracellular electrodes in R_{1-6} (Fig. 1 in Minke et al., 1975); and between the interpretations of these phenomena (ERG evidence based on spectral sensitivity measurements: Cosens and Wright, 1975; ERG evidence based on data from morphological mutants reported in Harris et al., 1976; Stark, 1975; ERG evidence and intracellular recordings: Minke et al., 1975). These similarities strongly suggest that both techniques yield measures of the same transmembrane events which occur in R_{1-6}. A considerable amount of evidence is accumulating which indicates that the light-induced depolarisation of invertebrate photoreceptors is caused by an influx of sodium which results from a light-induced increase in permeability to this ion across the transducing membrane (e.g. see Wulff et al., 1975 for references to work on *Limulus*). In addition, Brown and Cornwall (1975) have recently shown that the PDA observed in *Balanus* photoreceptors is accompanied by prolongation of such light-induced permeability. This evidence leads to the hypothesis that a PDA results from the failure of membrane conductance channels to close following an appropriate stimulus. On this interpretation the fast and slow decline in the transmembrane afterpotential of R_{1-6} (seen in the ERG or via intracellular electrodes) must reflect differences in the rate of closure of these channels.

A convincing explanation of these differences in the rate of dark-closure of the conductance channels (and hence an explanation of both transduction and the PCNA/PDA phenomena) may be constructed around the 'excitor-inhibitor' hypothesis proposed to explain the PDA observed in *Limulus* and *Balanus* (Hochstein et al., 1973; Minke et al., 1973); and there are, in addition, theoretical reasons for believing that transmitters of some kind are involved in the excitation of invertebrate photoreceptor membranes (for a review see Cone, 1973). However, as we have noted, the hard corroborating evidence for an 'excitor-inhibitor' interaction in R_{1-6} in *Drosophila* is lacking. The principal point of departure between the behaviour of R_{1-6} in *Drosophila* and of the *Balanus* and *Limulus* photoreceptors is that we were unable to demonstrate a disparity between the dark-decline of the PCNA of a fully blue-adapted eye and changes in the photopigment composition. Our data (Fig. 10b and text) confirm that the blue-adapted state persists for several hours in *Drosophila* (sensitivity data from the ERG: Cosens and Briscoe, 1972; duration of PCNA and PDA: Minke et al., 1975): the duration of the PCNA/PDA in *Drosophila* contrasts markedly with the brevity of the PDAs reported in other species. The fact that large afterpotentials are measurable several hours after blue-adaptation and that a further blue stimulus will again blue-adapt the eye of *Drosophila* (i.e. reduce the response amplitude and increase the size of the PCNA: Fig. 10b and text) argues that the decline of the afterpotential in this species accompanies the decay of M580, or outlasts the changes in pigment composition. The latter of these is unlikely since an orange stimulus after several hours in the blue-adapted state will again orange-adapt the eye (i.e. eliminate the PCNA and restore the size of the response amplitude: Fig. 10b and text). The data thus confirm the long time-constant for the dark-decay of M580 (6 h: Pak and

Lidington, 1974) and it appears that in *Drosophila* the decline of the PCNA/PDA is dependent upon the decay of M580. It follows that there is a component of membrane conductance which is correlated with the number of M580 molecules which are present in the receptor membranes of R_{1-6}.

Our hypothesis that there is a component of membrane conductance in R_{1-6} of *Drosophila* which is correlated with the number of M580 molecules present in the photoreceptor membrane is supported by the following additional evidence.

(1) The phenomena of blue and orange adaptation are overt in white-eyed *Drosophila* (Cosens and Briscoe, 1972; Cosens and Wright, 1975; Stark, 1975; Minke et al., 1975) but not readily observed in the red-eyed wild-type (Cosens and Briscoe, 1972): in vivo and in vitro studies of the visual pigments of white-eyed *Drosophila* reveal that blue light causes the production of M580 while orange light eliminates M580 and regenerates R480 (Ostroy et al., 1974; Pak and Lidington, 1974; Harris et al., 1976), similar conversions have not been reported for the red-eyed fly.

(2) The effects of blue and orange adaptation are cumulative (Figs. 1, 2, 3, 4, 5, 8 and 9) as are the accumulation (using blue-light) and the elimination (using orange light) of M580 in white-eyed *Drosophila* (Harris et al., 1976).

(3) White-eyed *Drosophila* with reduced visual pigment show reduced PCNAs (Stark and Zitzmann, 1976); a similar finding in respect of the PDA is visible in the records from *Calliphora* (Razmjoo and Hamdorf, 1976).

A correlation between a component of membrane conductance and the concentration of M580 in R_{1-6} neither validates nor refutes the hypothesis of an 'excitor-inhibitor' interaction forming the basis of transduction in these cells in *Drosophila*. It does imply that 'excitors' *if present*, have a lifetime exceeding that of M580 (that is, they have a time-constant of decay of greater than 6 h), or, alternatively, that 'excitors' latch open certain conductance channels which remain open until they are unlatched by 'inhibitors'. Whatever the underlying mechanism the persistent conductance increase associated with the presence of M580 and the resulting reduction in measured response amplitude (the afterpotential effect: Figs. 2, 7 and text) may account for the 'supression' of rhodopsin by metarhodopsin reported in *Calliphora* (Razmjoo and Hamdorf, 1976).

Interpreting the Afterpotential Effect

The interpretation of the observed correlations between response amplitude and ERG afterpotential (Figs. 2 and 7) depends on what exactly the ERG is measuring. If, as we have already suggested, the changes visible in the ERG of white-eyed *Drosophila* during blue and orange adaptation reflect transmembrane events in R_{1-6}, then calculations using the type of analogue of the transducing membrane described by Shaw (1968) should account for the afterpotential effect. Shaw's type of analogue has gained considerable respect in the interpretation of intracellular data (e.g. see Laughlin, 1975) but does not seem to have been applied to ERG data. The model assumes that the light-

induced conductance across the transducing membrane is proportional to light intensity, and predicts a sigmoid relationship between transmembrane potential and the log of stimulus intensity (Shaw, 1968). Calculations using this type of analogue reveal an expected curvilinear negative correlation between the response to a fixed intensity stimulus and the afterpotential produced by an independent source of conductance, and that this negative correlation becomes increasingly linear as the intensity of the stimulus is increased: which is exactly the relationship reported in the results section (e.g. see Fig. 5 and accompanying text). It also follows, directly, from the model that an exponential decline of conductance results in a sigmoid decline in afterpotential: we observe sigmoid declining afterpotentials when M580 is eliminated exponentially (Figs. 4, 5, 6). It appears that in the white-eyed *Drosophila* (where the retinula cells of all of the ommatidia tend to respond in concert) the membrane analogue will describe data from the ERG, as it does data from intracellular recordings (Shaw, 1968; Laughlin, 1975). It is implied that, at least in the particular experimental situation of blue/orange adaptation, the ERG of white-eyed *Drosophila* is revealing events which occur across the transducing membrane of photoreceptor cells.

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